European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

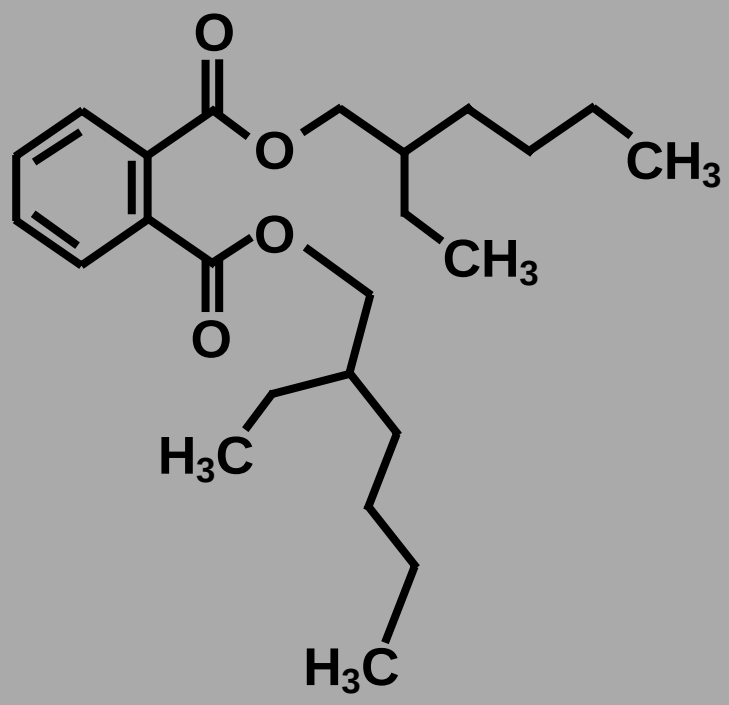
Existing Substances

European Union Risk Assessment Report

CAS No: 117-81-7

EINECS No: 204-211-0

bis(2-ethylhexyl)phthalate (DEHP)



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European Union Risk Assessment Report
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The mission of the IHCP is to provide scientific support to the development and implementation of EU polices related to health and consumer protection. The IHCP carries out research to improve the understanding of potential health risks posed by chemical, physical and biological agents from various sources to which consumers are exposed.

The Toxicology and Chemical Substances Unit (TCS), commonly known as the European Chemicals Bureau (ECB), provides scientific and technical input and know-how to the conception, development, implementation and monitoring of EU policies on dangerous chemicals including the co-ordination of EU Risk Assessments. The aim of the legislative activity of the ECB is to ensure a high level of protection for workers, consumers and the environment against dangerous chemicals and to ensure the efficient functioning of the internal market on chemicals under the current Community legislation. It plays a major role in the implementation of REACH through development of technical guidance for industry and new chemicals agency and tools for chemical dossier registration (IUCLID5). The TCS Unit ensures the development of methodologies and software tools to support a systematic and harmonised assessment of chemicals addressed in a number of European directives and regulation on chemicals. The research and support activities of the TCS are executed in close co-operation with the relevant authorities of the EU Member States, Commission services (such as DG Environment and DG Enterprise), the chemical industry, the OECD and other international organisations.

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RISK ASSESSMENT

Cataloguing data can be found at the end of this publication

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CAS No: 117-81-7

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RISK ASSESSMENT

Final Report, 2008

Sweden

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Review of report by MS Technical Experts finalised:	**September 2005**
Final report:	**2008**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

Roland Schenkel
Director General
DG Joint Research Centre

Mogens Peter Carl
Director General
DG Environment

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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 117-81-7
EINECS Number: 204-211-0
IUPAC Name: bis(2-ethylhexyl) phthalate

Environment

Atmosphere

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion is reached because:

- the risk assessment shows that risks are not expected. Risk reduction measures already being applied are considered sufficient.

Aquatic ecosystem

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of:

- concern for birds consuming mussels exposed to DEHP near sites processing polymers with DEHP or sites producing printing inks, sealants and/or adhesives with DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

Conclusion (i) There is a need for further information and/or testing.

Further refinement of the assessment may remove some concern. This conclusion is reached because of:

- concern for sediment dwelling organisms as a consequence of exposure to DEHP near sites processing polymers with DEHP or sites producing lacquers, paints, printing inks, sealants and/or adhesives with DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for other environmental spheres will eliminate the need for further information on sediment dwelling organisms.

Terrestrial ecosystem

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of:

- concern for mammals consuming earthworms exposed to DEHP near sites processing polymers with DEHP or sites producing lacquers, paints, printing inks, sealants and/or

adhesives with DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

Conclusion (i) There is a need for further information and/or testing.

Further refinement of the assessment may remove some concern. This conclusion is reached because of:

- concern for soil organisms exposed to DEHP near sites processing polymers with DEHP or sites producing printing inks, sealants and/or adhesives with DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for other environmental spheres will eliminate the need for further information on soil organisms.

Micro-organisms in the sewage treatment plant

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion is reached because:

- the risk assessment shows that risks are not expected. Risk reduction measures already being applied are considered sufficient.

Human Health

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of:

- concerns for testicular effects, fertility, toxicity to kidneys, on repeated exposure and developmental toxicity as a consequence of inhalation and dermal exposure during production, processing and industrial end-use of preparations or materials containing DEHP

Consumers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of:

- concerns for children with regard to testicular effects, fertility, and toxicity to kidneys, on repeated exposure as a consequence of oral exposure from toys and child-care articles, and multiple routes of exposure.
- concerns for children undergoing long-term blood transfusion and neonates undergoing transfusions with regard to testicular toxicity and fertility, as a consequence of exposure from materials in medical equipment containing DEHP.

- concerns for adults undergoing long-term haemodialys with regard to repeated dose toxicity to kidney and testis, fertility, and developmental toxicity, as a consequence of exposure from materials in medical equipment containing DEHP.

Humans exposed via the environment

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of:

- concerns for children with regard to testicular effects, fertility, and toxicity to kidneys, on repeated exposure as a consequence of exposure via food locally near sites processing polymers with DEHP, or sites producing sealants and/or adhesives, paints and lacquers or printing inks with DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.
- concerns for children with regard to testicular toxicity, as a consequence of exposure via food grown locally near sites recycling paper or municipal sewage treatment plants. The scenarios that give concern are generic scenarios based on default emission data.

Human Health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion is reached because:

- the risk assessment shows that risks are not expected. Risk reduction measures already being applied are considered sufficient.

CONTENTS

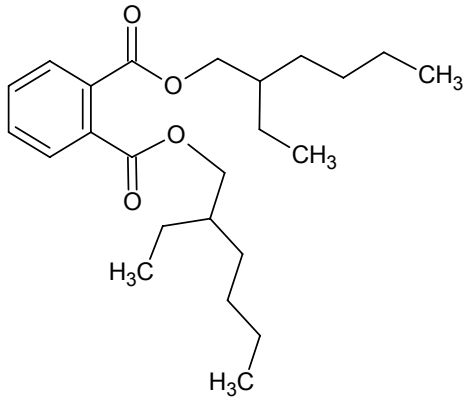
EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 117-81-7
EINECS Number: 204-211-0
IUPAC Name: Bis(2-ethylhexyl) phthalate
Molecular formula: $C_{24}H_{38}O_4$
Structural formula:



Molecular weight: 390.6
Synonyms: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
Bis(2-ethylhexyl) 1,2-benzenedicarboxylate
Bis(2-ethylhexyl) o-phthalate
Bis(2-ethylhexyl) phthalate
DEHP
Di(2-ethylhexyl) phthalate
Dioctyl phthalate
DOP (pseudo-synonym, incl. also other isomeric forms of the alcohol part)
Phthalic acid dioctyl ester
Phthalic acid, bis(2-ethylhexyl) ester

Trade names:		
	FLEXOL Plasticizer DOP	Union carbide
	Essochem DOP	ESSO
	Palatinol AH	BASF AG
	Palatinol AH-L (med.) 99.5%	BASF AG
	Genomoll 100 99.7%	Hoechst AG
	Vestinol AH 99.5%	Hüls AG 1994a

1.2 PURITY/IMPURITIES, ADDITIVES

Purity/impurity

Only few data about the purity are available. This indicates a high purity level (99.7%). The impurities found are mainly other phthalates (Hüls AG 1995g).

Additives

"Bisphenol A"; 4,4'-isopropylidenediphenol (synonyms e.g.: diphenylolpropane; 2,2-bis(p-hydroxyphenyl)-propane). CAS No. 80-05-7. Some customers request this additive in the range of 0.025 to 0.5% (ECPI 1998a).

1.3 PHYSICO-CHEMICAL PROPERTIES

Physical state

DEHP is a colourless oily liquid at normal temperature.

Melting-point

-50°C (Sorbe 1984, in Rippen 2005), –55°C (pour point, BUA 1986; CRC 1995)

Boiling-point

Approximately 230°C at 5 mm Hg (BASF1994b; Clayton 1981, in HSDB 2005)

385°C at 1013 hPa (Verscheuren 1983 and Sorbe 1984 in Rippen 2005)

Density

0.984 g/cm^3 at 20°C (OECD 1979, in Rippen 2005)

0.986 g/cm^3 at 20°C (IARC 1982, in HSDB 2005)

0.980-0.985 g/ml (Furtmann 1996)

Vapour pressure

A large range of values on the vapour pressure is available in the literature (0.00000004 - 0.0014 Pa, Staple et al. 1997b). However, recent studies have shown that many of these values probably are overestimations due to interference from impurities (Rippen 1992). In a newly made measurement with 99.5% pure DEHP, the vapour pressure was estimated to 0.000034 Pa at 20°C (See Table below) (Hüls AG 1997). This value is used for assessing the environmental fate.

°C	Pa	Comment
10	0.000010*	
15	0.000023	
20	0.000034*	used in EUSES / fugacity
30	0.00013*	
40	0.00047*	
50	0.0016*	
60	0.0057	

70	0.011	max. indoor car (BUA 1986)
80	0.039	
90	0.10	
100	0.29	
110	0.76	
120	1.9	
140	4.5*	
160	2.4*	injection moulding, PVC
180	80*	
203	287	
210	389	
216	511	

* Extrapolated values

Water solubility

A large range of values on the water solubility is available in the literature (0.003-1.3 mg/l at 20-25°C) (see **Table 1.1**). A probable explanation of this is that DEHP easily forms more or less colloidal dispersions in water, which increase the amount DEHP in the water phase (Lundberg and Nilsson 1994). The colloidal formation might be of relevance in understanding laboratory studies in aquatic media. A colloidal water solubility of about 0.34 mg/l (ECETOC 1985) is assumed in this assessment. However, the non-colloidal solubility is more relevant to the fugitive long-term distribution in the environment. Staples et al. (1997) reviewed present studies on water solubility and compared these with different calculated values. Based on these they suggested a non-colloidal solubility of 0.003 mg/l(the temperature for this solubility was not mentioned). An alternative method to get round the colloidal disturbance was developed by Thomsen et al. 2001. By measuring the surface tension the solubility in water was estimated to be about 0.017 mg/l at 22°C. The authors suggest that the deviation from earlier estimations based on slow stirring technique may depend on high affinity for adsorption at interfaces of laboratory equipment. This then may reduce the accuracy in the recovery of DEHP. Available studies clearly show considerable difficulties in estimating relevant water solubility. In a bioaccumulation study on fish (Mehrle and Mayer, 1976;Mayer 1976) made at different concentration between 1.9 to 62 µg/l a sharp reduction in the BCF was observed between 4.6 and 8.1 µg/l (see **Table 3.44**). A probable explanation is that at test concentrations over 4.6 µg/l DEHP is dosed over the non-colloidal water solubility (assuming a lower bioavailability for colloidal/precipitated DEHP). Based on these data the value of 3 µg/l suggested by Staples et al., is used in the risk assessment.

Natural constituents in water may influence the solubility. One study identifies a reduction of the solubility with 15% in well water and 42% in seawater compared to distilled water (Howard et al. 1985). Based on fundamental chemistry the water solubility for DEHP can be assumed to increase in presence of natural occurring dissolved organic matter. Studies describing this are,

however, not available. Monitoring studies indicate that DEHP may occur at higher concentrations (e.g. 92 µg/l in a ground water sample near an industrial landfill, Branzén 2000).

Partition coefficient; n-octanol/water

Log Kow values from 4.8 to 9.6 are available (see **Table 1.1**). Due to the ability of DEHP to form a colloidal dispersion in water, several reported values of log Kow probably are underestimations (Howard et al. 1985). Only results based on HPLC technique or slow-stir methods are therefore assumed to be useful. A value of 7.5 is recommended in a recent review (Staple et al. 1997b). This value is used in the assessment. In the EUSES model the value 7.0 is used (the highest recommended value).

Table 1.1 Available values on water solubility and log Kow on DEHP

Chemical and Physical properties	°C	Comment (Reference)
Water solubility (mg/l)		
0.0006	25°C	Sea water, "generator column" method (Staples et al. 1997)
0.0011 (calc.)	-	(Staples et al. 1997)
0.0026 (calc.)	-	(Staples et al. 1997)
0.003	?	Review recommendation. Used in this assessment (Staples et al. 1997)
Approximately 0.007-0.04	20°C	(BASF AG 1994b)
0.017	22°C	Surface activity method (Thomsen et.al. 2001)
0.029	20°C	(Rippen 1992)
0.041	20°C	(Leyder et al. 1983)
0.045	?	(BASF AG 1989)
0.046	20°C	(OECD 1981)
0.0466	25°C	(ECETOC 1985)
0.0476	15°C	(ECETOC 1985)
0.16	?	Sea water (Howard et al. 1985)
0.28	?°C	(ECETOC 1985)
0.34	25°C	(Howard et al. 1985)
0.34	?	Colloidal suspension (ECETOC, 1985)
0.4	20°C	(ECETOC 1985)
0.6	?°C	(ECETOC 1985)
1.16	?	In sea water (Staples et al. 1997)
1.2	?	In sea water (Staples et al. 1997)
1.3	?°C	(ECETOC 1985)
Solubility ratio:1.0: 0.85: 0.58	?	Distilled water: well water: salt water (Howard et al. 1985)

Table 1.1 continued overleaf

Table 1.1 continued Available values on water solubility and log Kow on DEHP

Chemical and Physical properties	°C	Comment (Reference)
log Kow		
Approximately 4.8	?	(BASF AG 1994b)
4.88	25°C	(Batelle Institut 1982) (BUA 1986)
4.66-5.45	?	Average: 5.11 (Howard et al. 1985)
5.03	?	Calculated (Hoechst AG 1993)
7.14 ± 0.15	?	(Brooke et al 1990)
7.27	?	Measured (Ellington and Floyd 1996)
7.45 ± 0.06	?	Slow-stir apparatus (De Bruijn et al 1989)
7.5	-	Recommend. Used in this assessment(Staples et al. 1997)
7.7	20°C	Measured, RP-HPLC (Condea 1995)
7.86	?	Measured, +- 1.33 (Klein et al. 1988)
7.94	?	"Overestimation", HPLC-method (Howard et al. 1985)
8	20°C	(BASF AG 1987)
9.64	20°C	Calculated (Leyder et.al. 1983)

Other Physico-chemical properties

Surface tension

32.2 mN m^{-1} at 20°C (Marwedel 1966, in Rippen 2005)

Granulometry

Not applicable (liquid)

Flash point

200°C (DIN 51758) (Riddik et al. 1986)

Autoflammability

Ignition temperature: 370°C (BASF AG 1994a)

Explosivity

Explosion limit: 0.15-0.18 vol.% (BASF AG 1994a; Sorbe 1984 in Rippen 2005)

Explosion limit: 0.3-49 vol.% (Auergesellschaft 1989, in Rippen 2005)

Oxidising properties

Based on DEHP's chemical structure this should be low. "Hazardous reaction influenced by: strong oxidising agents" (Riddik et al. 1986).

Table 1.2 Summary of physico-chemical properties

Property	Value	Reference
Physical state	Colourless oily liquid	see above
Melting point	-55°C or -50°C	see above
Boiling point	230°C at 5 mm Hg 385°C at 1013 hPa	see above
Density	0.986 or 0.984 g/cm^3 at 20°C 0.980-0.985 g/ml	see above
Vapour pressure	0.000034 Pa @ 20°C	see above
Water solubility	3 µg/l @ 20°C	see above
Partition coefficient n-octanol/water (log value)	7.5	see above
Granulometry	N.R:	see above
Conversion factors air	1 ppmv = 16.2 mg/m3 at 20°C and 1013 hPa	Auergesellschaft 1989 in Rippen 2005
Flash point	200°C	see above
Autoflammability, ignition temperature	370°C	see above
Explosive properties	0.15 - 0.18 vol.% 0.3 – 49 vol%	see above
Oxidizing properties	Not strong oxidising agent	see above
Viscosity, dynamic	81 mPa s at 20°C 58 mPa s at 25°C	IARC 1982 in Rippen 2005 Frische 1979 in Rippen 2005
Henry's constant	see section 3.1.2.2.2	

1.4 CLASSIFICATION

1.4.1 Current classification

Human health:

Classification according to Annex I: Toxic to reproduction, Category 2; R60-61

Environment:

Classification according to Annex I: None

1.4.2 Proposed classification

Environment

None

Human Health

As adopted in the current classification (above); as given in the 28th ATP of Directive 67/549/EEC.

2 GENERAL INFORMATION ON EXPOSURE

2.1 GENERAL

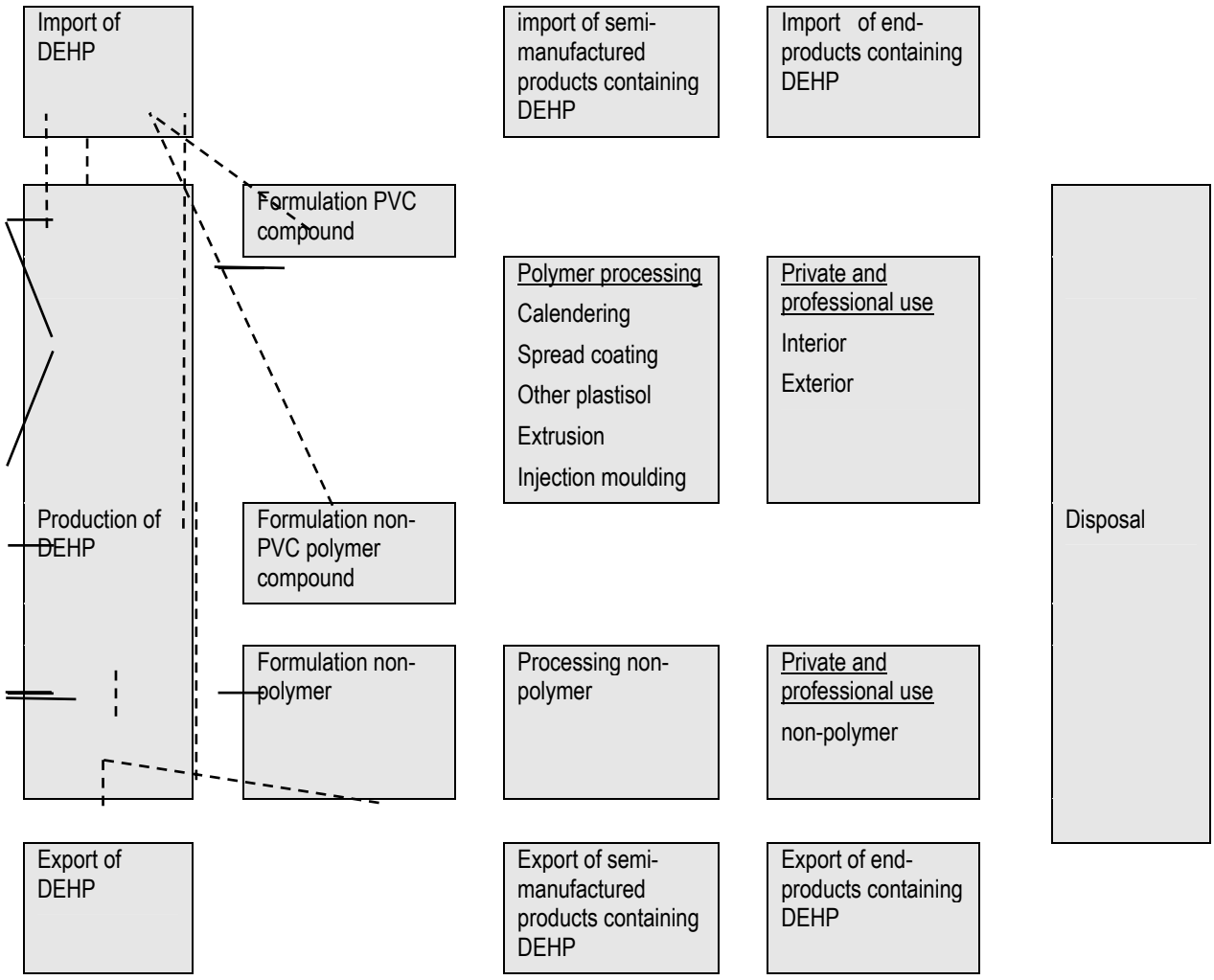
The substance di(2-ethylhexyl) phthalate (hereafter referred to as DEHP) is widely used as a plasticiser in polymer products, mainly PVC. Plasticisers have the function of improving the polymer material's flexibility and workability. DEHP is one of a number of substances used as plasticiser in PVC and other polymer materials. Examples of other plasticisers are other phthalates, adipates, trimellitates, and phosphates (Kroschwitz, 1998).

The content of DEHP in flexible polymer materials varies but is often around 30% (w/w) (Kroschwitz, 1998). Flexible PVC is used in many different articles e.g. toys, building material such as flooring, cables, profiles and roofs, as well as medical products like blood bags, dialysis equipment etc. DEHP is used also in other polymer products and in other non-polymer formulations and products. Consequently the wide use of DEHP gives rise to many possible scenarios of human and environmental exposure.

Information is available identifying life-cycle stages for production, formulation, processing, use, transport/storage, waste/disposal and emissions of DEHP to the environment. European manufacturers have been identified and the volumes of DEHP production, use, and import are detailed.

The general information on manufacture and use of DEHP as a PVC plasticiser is envisaged to be well documented. However, information on the use of DEHP in formulation and processing of PVC polymers by down-stream industries (off-site; small industries) is not available with respect to the number and size of the sites. Furthermore, it is not known whether the import volumes are representative. It may be that the import volumes are underestimated. In addition, there is no detailed information on formulation/processing of DEHP in non-PVC polymers or in non-polymer uses, neither on the use of these products. Error! Reference source not found. gives an overview of relevant life cycle stages for DEHP.

Figure 2.1 Overview of the relevant life cycle stages of DEHP



dotted line is import and export

2.2 TONNAGE, PRODUCTION, CONSUMPTION

The quantities given in this report originate from industrial sources from different years, using generic terms like “Western Europe” and “Europe” etc. The quantities may be valid for different single years within the first seven years of the 1990´s (1990-1996).

Some information on quantities is given as the total quantity of all phthalates. The amount of DEHP has been calculated, assuming the DEHP part of total phthalate plasticisers to be 51% (ECPI, 1996a). The quantities of DEHP in non-PVC polymer use and in non-polymer use are calculated from assumed percentages of the total use. This gives a rough estimate of the amounts of DEHP in different life cycle stages. It is assumed that the volumes reported are representative for the EU and that variation between different years is insignificant.

The production volume of DEHP in Western Europe for 1997 is 595,000 tpa (ECPI, 1998), which is the value used in this assessment.

Europe is a significant producer and exporter of plasticisers. In 1993 manufacturers in Europe produced 46% of the world demand of plasticisers and consumed 37% (Cears and Poppe 1993). According to ECPI, the consumption was 476,000 tpa in 1997, which is used in this assessment. According to the industry, the export of DEHP from the EU in 1997 was 186,000 tonnes. It was expected to be less in 1998 (Rykfors, 1998-06-05).

The import can be calculated: Import= Consumption + Export – Production = 476,000 + 186,000 –595,000 = 67,000 tpa. This figure is used in this assessment. Two companies, which are importing DEHP to the EU, are registered in IUCLID, one importing amounts between 1,000 and 5,000 tpa and another importing between 10,000 and 50,000 tpa.

The global production of DEHP was estimated to be between 1 and 4 million tpa, 1994 (Klöpffer, 1994 cited in Huber et al., 1996). The worldwide consumption of plasticisers is estimated at 3.5 million tpa (Cears and Poppe, 1993). The production of DEHP in Japan was 348,600 tonnes in 1993. The import during the same period was 17,400 tonnes (MITI, 1997).

Phthalates belong to a group of substances that accounts for 92% of the consumption of plasticisers in Western Europe. The use of DEHP corresponds to 51% of the total phthalate consumption used as plasticiser in EU (ECPI, 1996a). The mean annual plasticiser consumption 1990-1995 in Western Europe was about 970,000 tpa. 894,000 tpa of this was phthalates. For the entire plasticiser consumption in Western Europe (phthalates and others) approximately 90% are used in PVC.

There is no information on the amounts of DEHP imported into or exported from the EU in polymer products (and semi-manufactured products) or in other products. This creates uncertainty in the quantification of emissions and exposure.

The import of articles made of PVC to Sweden has been investigated in a KemI-report (KemI, 1996). The main “countries of origin”, for most groups of articles, are within the European countries. An exception is clothes made of plastics e.g. PVC, which are imported mainly from Asia. Another example is the Swedish import of plastic high boots. The import of plastic high boots from countries outside the EU was due to Swedish customs statistics 60-69% of the total import of this product group. This might not be representative for all countries in the EU or the EU as a unit and the situation is probably different for other product groups.

However, from this information we assume that the main amount of articles containing DEHP consumed in Europe are produced in Europe, but for some product groups the imported amount are significant. No data on the export of articles containing DEHP have been submitted, but we assume the export to be in the same order of magnitude as the import.

2.3 PRODUCTION

2.3.1 Production processes

Apparently all manufacturers of phthalate esters use similar processes. DEHP is produced by the esterfication of phthalic anhydride with 2-ethyl-hexanol. This reaction occurs in two successive steps. The first reaction step results in the formation of monoester by alcoholysis of phthalic acid. This step is rapid and proceeds to completion. The second step involves the conversion of the monoester to the di-ester. This is a reversible reaction and proceeds more slowly than the first. To shift the equilibrium towards the di-ester, the reaction water is removed by distillation. Elevated temperatures and a catalyst accelerate the reaction rate. Depending on the catalyst used, the temperature in the second step varies from 140°C to 165°C with acid catalysts and from 200°C to 250°C with amphoteric catalysts. Variations in purity may occur depending on catalyst, reactant alcohol and process type.

Excess alcohol is recovered and recycled and DEHP is purified by vacuum distillation and/or activated charcoal. The reaction sequence is performed in a closed system. This process can be run continuously or batchwise. Production of a particular phthalic ester may in some cases be conducted on a campaign basis (Hüls, 1995; King, 1996). Production plants often produce DEHP only due to the large volumes. A typical number of production days during a year is >330 days (Rykfors, 1998-06-05).

2.3.2 Production capacity

Site specific data on the number and location of sites producing DEHP in the EU have been submitted by industry, see **Table 2.1**.

Table 2.1 Companies producing and/or importing DEHP (IUCLID, ECPI, 1998)

Company		Location	Reported production year in IUCLID, ECPI 1998 (*), and ECPI, 2000 (#)
BASF AG		Ludwigshafen, Germany	91, 94*, 97*, 98#
BASF, Espanola		Tarragona, Spain	90, 94*, 97*, 98#
BP Chemicals		Hull, UK	92, 94*, 97*, 98#
BSL	Ceased 1996	Schkopau, Germany	94*, 98#
Celanese1		Oberhausen, Germany	92, 94*, 97*, 98#
Driftal GPD		Lisbon, Portugal	94*, 97*, 98#
Elf Atochem Fr		Chauny, France	94*, 94, 97*, 98#
Industrie Generali SpA,		Samarante, Italy	(more info. will come)

Table 2.1 continued overleaf

Table 2.1 continued Companies producing and/or importing DEHP (IUCLID, ECPI, 1998)

Company		Location	Reported production year in IUCLID, ECPI 1998 (*), and ECPI, 2000 (#)
Lonza 3		San Giovani, Valdarno, Italy	93, 94*, 97*, 98#
Lonza5 Enichem SpA	Ceased	Porto Marghera,Italy	93, 94*, 97*, 98#
Neste Oxo		Stenungsund, Sweden	92, 94*, 97*, 98#
Neste Oy		Porvoo, Finland	93, 94*, 98# (more info. will come)
Oxeno Olefinchemie2		Marl, Germany	92, 94*, 97*, 98#
Plastificantes de Lutxana (PDL)		Baracaldo, Spain	97*
SISAS	Ceased 1996	Pioltello, Italy	94*, 94**, 95**, 96**, 97**, 98** , 98#
SISAS Pantochim		Feluy, Belgium	94*, 97*, 94**, 95**, 96**, 97**, 98**, 98#
SISAS4		Ostend, Belgium	91, 94*, 97*, 94**, 95**, 96**, 97**, 98**, 98#
ÖMV, Chemie, Linz	Ceased 1993	Linz, Austria	90, 91, 92, 93, 98#

* From ECPI, 1998a
** From SISAS, 1999
\# From ECPI, 2000
1 Name changed from Hoechst in 1997
2 Name changed from Hüls in 1997
3 Name changed from Alusuisse in 1994
4 Name changed from UCB in 1995
5 Lonza is carrying on production of DEHP at the Enichem plant at Porto Marghera
6 Sintetica SpA is now the SISAS plant at Piotello and it closed 1996

2.3.3 Transport and storage

Almost all the phthalates consumed in Western Europe are distributed via road tankers. The actual filling of a road or rail tanker typically takes 20 to 40 minutes. The filling of road and rail tankers is carried out at gantries, which are largely in open air. The filling of drums is on the other hand carried out in-house mainly by automated facilities. Only a low percentage of the produced phthalates are distributed in drums.

Approximately 130 ktpa plasticisers are transported by ship within Europe (ECPI, 1996b). If DEHP constitutes 47% of all plasticisers the amount of DEHP transported by ship is 61 ktpa.

2.4 USES

2.4.1 Introduction

For convenience the industrial use and end-use of DEHP can be divided into three main product groups:

I) PVC,
II) non-PVC polymers,
III) non-polymers.

Around 97% of DEHP is used as plasticiser in polymers, mainly PVC (97/100 · 476,000 tpa = 462,000 tpa). Examples of PVC products that contain DEHP are given in **Table 2.2**. Detailed

information on volumes used in different products is only available for PVC applications (ECPI, 1996a, ECPI, 1996b). Between two and three per cent is used in other polymers e.g. other vinyl resins and cellulose ester plastics[4]. In this risk assessment, all polymer products are hereafter regarded as "PVC-products". Approximately 78% of this volume is estimated to be used in indoor applications and the remaining approximately 22% in outdoor applications (see **Table 2.2**). The uses outdoors are divided into different applications.

Additionally, about three per cent (14,280 tpa) is used for non-polymer applications (ECPI, November 1999) such as adhesives and sealant, lacquers and paints, printing inks for paper and plastics, printing inks for textiles, rubber and ceramics for electronic purposes. Another use is as dielectric fluid in capacitors (WHO, 1992), with one producer selling 40 tpa for this application (Rykfors, 1998-06-05).

The group non-polymer uses is inhomogeneous and includes some applications with polymers in the products e.g. in adhesives/sealant and in paints.

Non-polymer uses include additives for advanced ceramic materials (Neste, 1996). Advanced ceramic materials are used in electronic and structural applications. Structural ceramics are defined as stress-bearing components or ceramics coatings for stressed parts. Applications for these include, for example, industrial thermal processing equipment and energy intensive consumer appliances.

Raw material for advanced structural and electronic materials include inorganic oxide powders, including alumina, zirconia, silica and barium titanates and ferrites, and organic additives, which make the powder easier to treat, but do not remain in the final product.

Dioctylphthalate and DIDP are in the group of the most popular plasticisers used in these advanced ceramic materials.

Table 2.2 Material types containing DEHP based on industry data (Industry, 1999). The polymer applications for outdoor use are divided in the different uses

Material	Distribution	DEHP (tpa)	Indoor uses (approximately78%)	Outdoor uses (approximately 22%)	
Polymer – total	97%	462,000	362,000		100,000
				Roofing material	1,000
				Roofing (coil coating)	5,000
				Cables	20,000
				Coated fabric	21,000
				Hoses and Profiles	6,000
				Car under-coating	7,000
				Shoe soles	40,000
Non-polymers	3%	14,280	****		
Total	**100%**	**476,000**			

**** No data available.

[4] Other polymers where DEHP are used are other vinyl resins and cellulose ester plastics cellulose nitrate, cellulose acetate butyrate, vinyl chloride/vinyl acetate copolymer with cellulose acetate butyrate, ethyl cellulose, polymethyl methacrylate, polystyrene, vinyl chloride/vinyl acetate copolymer (Gächter R and Müller H, 1990).

Information from the product registers in Sweden and Denmark supports the data on use pattern of DEHP provided by industry.

2.4.2 Industrial use

2.4.2.1 Polymers (PVC and non-PVC polymers)

According to information provided by industry, formulation and processing generally occur at the same site. However, DEHP may be formulated and processed at different sites but it is not known to what extent this occurs. An assumption made by industry (Rykfors, 1998) estimates that 15-25% of the total amount of DEHP used in Europe is formulated and processed to a semi-manufactured product (compound) at one site and processed at another place. Large producers of DEHP-containing products often produce the compound themselves, while smaller producers buy the compound with a specified formula (Cadogan D. pers. comm.).

"Formulation" is used in this assessment to denote a sequence of steps mixing DEHP into a polymer or into another material. Some examples of formulation are production of compounds and master-batches. "Processing" includes the forming/moulding of the DEHP-containing material.

EuPC is the professional representative body of plastic converters within Europe whose activity embraces all sectors of the plastics converting industry. EuPC represents over 37,000 predominantly medium-sized companies. The combined processing capacity of EuPC's members amounts to more than 35 million tonnes of plastic per year.

EuPC estimates that 6,500 companies are involved in the soft PVC value chain. Only about 800 of these are soft PVC converting plants relevant in the scope of this DEHP emission assessment. The other companies use intermediates, are fabricators, wholesalers and installers (EuPC, 2005).

2.4.3 Formulation

Typical formulations of DEHP in flexible PVC-products are 30% (w/w) (Kroschwitz, 1998).

According to UCD (1998) there are two major routes for plasticised PVC formulation. The resins used in these routes are chemically the same but may differ in physical form (particle shape etc.).

- "Dry-blending": The PVC resin is blended with plasticiser and other additives at a temperature of typically around 100 -120°C. Temperatures over 100°C occur occasionally. During dry blending the exposure of hot material to open air is small. A total charge of typically 150 kg, including 50 kg of plasticiser and a volume of air in the blender of 100 litres. At 100°C the saturation concentration of DEHP is 20 mg/m^3, i.e. 0.1 m^3 · 20 mg/m^3=2 mg. Assuming one air exchange per run, the amount of emitted DEHP vapour is very small. A dry powder is obtained (the so-called "dry blend"), which can be stored and shipped in bulk or bags. The hot blended powder material can be conveyed to a compounding extruder producing a semi-manufactured product. Further processing may occur via extrusion, injection moulding and sometimes calendering at the same or at another site downstream.
- "Plastisol blending": The PVC resin is blended with plasticiser and other ingredients at room temperature. Approximately 30-35% of all plasticiser in PVC is used in plastisol

applications. A pasty liquid (a so-called "plastisol" or "paste") results. Further processing may occur via spread-coating, dipping, spraying and various other moulding processes

A third method, rather obsolete, is the so-called Banbury blending or mixing. It is a batch process starting with the raw material at ambient temperature and subsequently increasing the temperature up to maximum of 120 -140°C.

2.4.4 Processing

PVC is processed in a number of ways: calendering, extrusion, injection moulding, several plastisol applications including rotational moulding, dip coating, slush moulding, spray coating and miscellaneous small to very small applications.

As is noted specifically for some processes, fume elimination equipment is commonly used to reduce emissions considerably, often by one order of magnitude. However, on smaller sites such equipment is less likely to be available.

The further processing of the "dry-blend" is performed by heating the blend in one or several stages e.g. by friction or by heating the surfaces and transferring it into the molten state.

The moulding occurs above 160°C.

Calendering

Calendering: running it through the gaps between the hot rolls of a calender (film, flooring, roofing). The average DEHP content in calendered products is about 25%.

Calendered flooring, roofing and wall covering is at least twice as thick as film and sheet and is produced at lower temperatures. As a consequence of the greater thickness, the surface to volume ratio and therefore the plasticiser loss is approximately half of that in film and sheet.

However, air purification equipment is more and more often used. For calendering this is mostly incineration, reducing the concentration.

Extrusion and injection moulding

Extrusion: pressing it through a die with subsequent cooling (hose, tubing, cable)

Injection moulding: pressing it into a "negative", cooled mould (boots, shoe soles)

The major different product types of plasticised PVC extrusion are "profiles" such as wire, cable and hose, and blow moulded film.

Exposure does not occur in the extruder it self, but occurs temporarily when the hot material leaves the dye. In addition, the surface to volume factor is much lower than in calendering.

Plastisol applications

The "plastisol" is applied by spreading on a substrate (e.g. paper or fabric) and thereafter heated (= "gelled" or "fused") to typically above 160°C. Major application modes are:

- spreading (spread coating). Paste is homogenised onto the tissue to be coated (flooring, coated fabric textile, woven glass) by a knife or a perforated roller. Spread coated products

are "fused" (gelled) in tunnel ovens heated with hot air at about 180°C. The energy is supplied by infrared heating source (IR) and/or hot air.

- dipping of moulds into plastisol. This is applied to the production of gloves.
- spraying or injection of pseudoplastic onto car body as anti-corrosive coating, or into crowns or capsules for beverage bottles
- slush and rotational moulding (fenders, car door arm rests, balls, dolls, boots, hollow articles). A spherical mould of required geometry is filled with the proper amount of paste, upon rotation due to centrifugal force the paste will be homogeneously spread over the inner walls of the mould. Gelation is accomplished by hot air and for large shapes by direct flame-heating.

In processes such as application of car undercoating and sealant, rotational moulding, dip coating and slush moulding, car undercoating is by far the largest volume application. In this process the sprayed coating is "dried" in long air-heated tunnel ovens at relatively low temperatures (130-160°C). The ovens in this industry invariably have integrated air incinerators and insignificant amounts of DEHP are emitted (ECPI, 1996b).

The phthalate polymer-plasticiser consumption for the different processing techniques (divided in groups of end-use products) is summarised in **Table 2.3** and shown in **Figure 2.2**. The figures are based on a marketing survey on the use of DEHP in Western Europe (Industry, 1999).

Table 2.3 Estimated amounts of DEHP for different applications and processes and the number of customers buying neat DEHP for each application in Western Europe 1997 (Industry, 1999)

Process	Application	Use of DEHP (tpa) in polymers	Number of customers
Calendering	Film/sheet and coated products Flooring, Roofing, Wall covering	71,400 34,748	74 20
	(Total, calendering)	106,148	94
Extrusion	Hose and profile	57,120	82
	Wire and cable Subtotal	80,920 138,040	62
	Compounding[1]	85,680	83
	(Total extrusion)	223,720	227
Injection moulding and extrusion (from compound)	Footwear and miscellaneous	83,680	?
Spread Coating	Flooring General (coated fabric, wall-covering, coil coating etc)	39,032 76,160	21 115
	(Total spread coating)	115,192	136

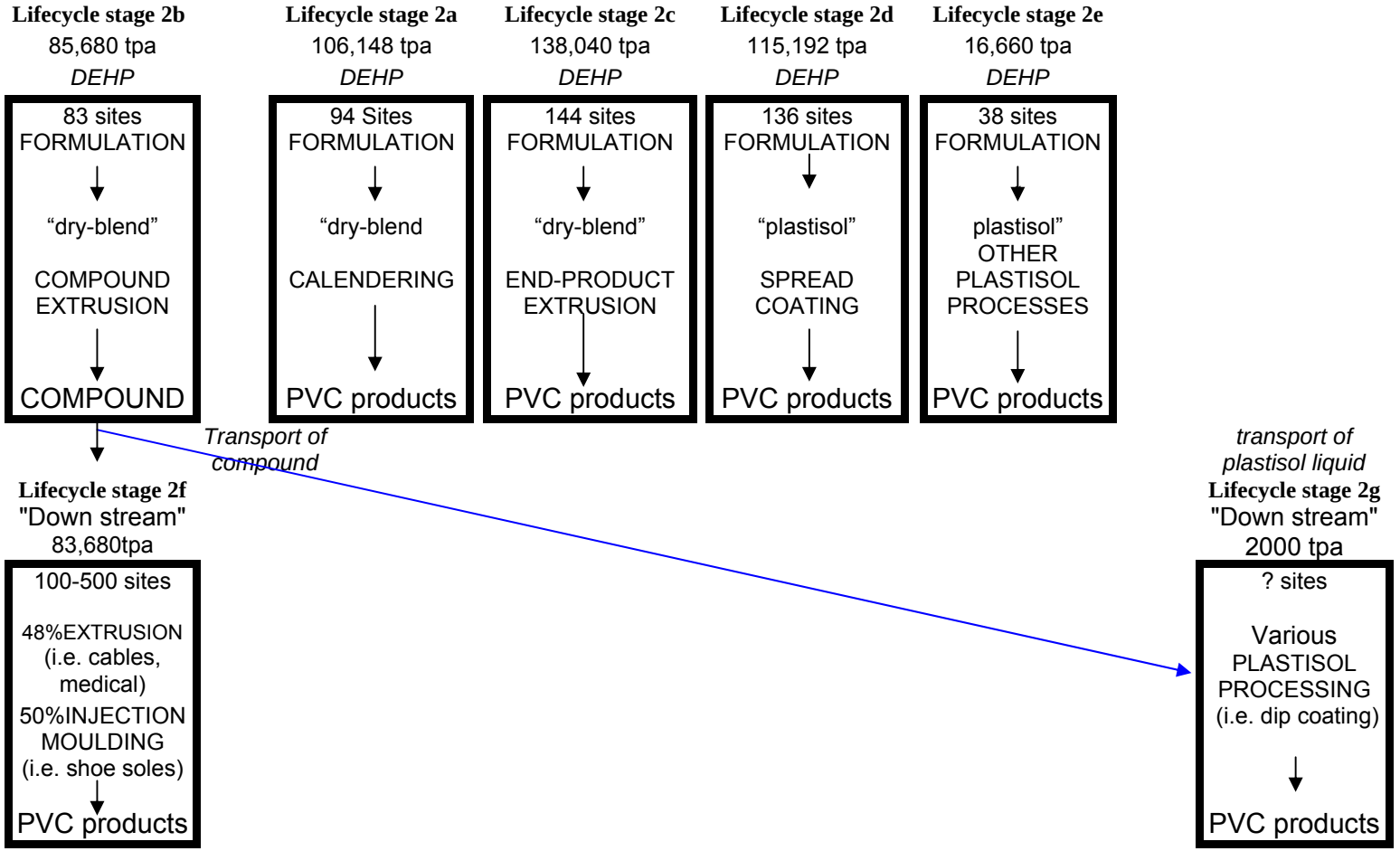
Table 2.3 continued overleaf

Table 2.3 continued Estimated amounts of DEHP for different applications and processes and the number of customers buying neat DEHP for each application in Western Europe 1997 (Industry, 1999)

Process	Application	Use of DEHP (tpa) in polymers	Number of customers
Other plastisols	Car undercoating Slush/rotational moulding, Dip coating	7,140 9,520	11 27
	(Total other plastisols)	16,660	38
Non-polymer applications[2]	Adhesives/sealant, rubber	11,142	
	Lacquers and paints	1,448	10
	Printing ink (paper and plastics)	1,661	10
	Ceramics	29	
	Total, non-polymer applications	14,280	
	Total, all uses of DEHP	476,000	

1) The semi-product (compound) is assumed to be produces by extrusion and to be used further for extrusion, injection moulding and plastisol applications at an unknown number of downstream users.
2) According to the market survey, 20 customers are buying DEHP with a amount of 2860 tpa for formulation of lacquers, paints and printing inks. In this assessment printing ink is defined as a separate scenario. Based on the Swedish Product Register Paints and lacquers is about the half of the volumes (2860/2=1,430 tpa). The market survey also identifies a use as ink for textiles of 210 tpa (no. of sites unknown). Due to its similar use this amount is added to the printing ink scenario (1,430+210=1640 tpa). No information is available of the number of formulation sites for these two scenarios. The number of 20 sites, however, is the total number of customers for these two scenarios and it is assumed that they are distributed equally (10+10) between the scenarios.
New data on the use of DEHP in ceramics gives that the quantity used in this application is about 29 tonnes per year. The quantity given in the market survey (Industry, 1999) is 210 tonnes per year. The new data on ceramics are used in the risk assessment. However, the given total amount used for non polymer applications in the market survey (14,280 tpa) will be used. The difference between the data on the use in ceramics (210-29=181 tpa) is proportionally shared among the remaining three groups of non-polymer applications, based on the relative distribution between them.

Figure 2.2 Different industrial uses of DEHP in polymers (1997)



The technical lifetimes for different polymer and non-polymer product groups containing DEHP are found in **Table 2.4**. The technical lifetime is the assumed average time span the article is in active use in the society.

Table 2.4 Technical lifetime for different polymer and non-polymer products containing DEHP (years)

Application	Technical lifetime (years)				
	ECPI[3]	Industry[1]	Miljö-styrelsen[2]	Other	Used in RAR
In-door application:					
Coated products	7	-	-	-	7
Film and sheets	7	-	1-5[5]	-	7
Hoses and Profiles	10[4]	-	1-10	-	10
Floors	10	-	-	20[6]	20
Sealant, adhesives etc	-	-	-	-	20
Cables	30	-	30-50	-	30
Lacquers and paints	-	-	-	-	7
Printing inks	-	-	-	-	1
Out-door application					
Roofing materials	-	20	-	-	20
Roofing (coil coating)	-	10	-	-	10
Cables	-	10-30	30-50	-	30
Coated fabrics	-	10	-	-	10
Hoses and Profiles	-	10	-	-	10
Car under-coatings	-	12	16	-	12
Shoe soles	-	5	-	-	5
Sealant, adhesives etc	-	-	-	-	20
Lacquers and paints	-	-	-	-	7

1) Industry, 1999
2) Miljöstyrelsen 1996 (Denmark)
3) ECPI 1996
4) Assumed to be the same as for flooring
5) PVC-foils.
6) Estimation of average age, Tarkett, 1999

The lifetimes for different PVC-products are reported to be 1-50 years (Miljöstyrelsen 1996). For film and sheets lifetimes of 1-7 years are reported. In this assessment 7 years is used. For hoses and profiles lifetimes of 1-10 years are reported. In this assessment 10 years is chosen. No data on the technical lifetime for sealants, adhesives etc. are found. A technical lifetime of 20 years is assumed as a reasonable worst case. For PVC in cars the lifetime was estimated to be 12-16 years. In this assessment 12 years is assumed to be more realistic. For roofing material Industry (1999) propose a lifetime of 20 years, which is also used in this assessment. For coil coating a lifetime of 10 years will be used in the assessment as proposed by ECPI, 1998b. For cables and wires the lifetime was estimated to be 10 - 50 years (Industry, 1999, Miljöstyrelsen, 1996). In this assessment 30 years is selected as an average. The technical lifetime for a building is assumed to be 100 year (expert judgement). No lifetime is available for fabric coating. However, it is assumed to be 10 years (Industry, 1999).

2.4.4.1 Non-polymer uses

No information on the formulation and processing of the non-polymer end-uses is available. However, generic environmental release estimations will be made.

2.4.5 Use of end-products

2.4.5.1 Polymers

No detailed information on the private and professional end-use is available. Some examples of flexible PVC end products containing DEHP:

- Insulation of cables and wires
- Profiles, hoses
- Sheets, film, wall- and roof covering
- Flooring
- Coatings and leather imitations (car seats, home furniture), shoes and boots, out-door and rainwear
- Pastes for sealing and isolation
- Plastisols e.g., car undercoating
- Toys and child-care articles (pacifiers, teething rings, squeeze toys, crib bumpers etc.)
- Medical products

2.4.5.2 Non-polymers

Little information has been found concerning non-PVC polymer and non-polymer uses of DEHP. However, DEHP is used among other plasticisers as an additive to rubbers, latex, mastics and sealant, inks and pigments, lubricants (ECPI, 1996a). Some examples of non-polymer end products containing DEHP:

- Lacquers and paints
- Adhesives
- Fillers
- Printing inks
- Dielectric fluids in capacitors
- Ceramics

2.5 RECOVERY AND DISPOSAL

Products containing DEHP may be material recycled into new products or disposed after the end-use. The disposed material may either be placed in a landfill or incinerated. A fraction of the disposed material is expected to remain in the environment after end use.

No information on recovery has been submitted.

2.6 RELEASES OF DEHP

2.6.1 General

Release of DEHP to the environment occurs during production, transport, storage, formulation and processing of PVC and non-polymers. Furthermore, plasticisers are not chemically bound to the matrix polymer in flexible PVC (or other materials). Therefore the plasticiser will to some extent be lost from the finished article during its use and after its final disposal.

Occupational exposure may occur at the production and at the industrial uses of DEHP (formulation, processing and at the industrial uses of end-products). Consumer exposure may occur via food and via medical products and use of end products. Exposure via food, water and air may occur as a result of emissions to the environment from all life cycle stages.

2.6.2 Environmental release scenarios (incl. indirect exposure of humans)

The life-cycle stages used in this assessment of environmental release are described below (see **Table 2.5**).

A new life-cycle stage has been introduced: "Waste remaining in the environment". This is assumed to be waste particles formed by weathering of polymer and non-polymer end product for outdoor use, such as roof coatings and car undercoating material.

Table 2.5 Description of scenarios related to different life-cycle stages used in the assessment of DEHP

Scenario	Tonnes DEHP	No. of sites	DEHP consumption (90%ile) [tpa]%	Note
1a) Production	595,000	12		
1b) Transport (included in No. 2)				
2) Formulation/processing of polymers				B, D
2a) Calendering	106,1486	94	2,824	
2b) Extrusion-compound	85,680	83	2,500	A
2c) Extrusion-products	138,040	144	2,500	
2d) Spread coating	115,192	136	1,500	
2e) Other plastisols + car undercoating	16,660	38	800	
2f) Injection moulding/extrusion	85,680	27+11	?	A, C
2g) Various plastisol applications	2,000	?	?	A

Table 2.5 continued overleaf

Table 2.5 continued Description of scenarios related to different life-cycle stages used in the assessment of DEHP

Scenario	Tonnes DEHP	No. of sites	DEHP consumption (90%ile) [tpa]%	Note
3-6) Formulation/processing/ of non-polymers:	14,280		?	B
3a) Sealants – Formulation	11,142	?	?	
3b) Sealants – Processing	11,142	?	?	
4a) Lacquers and paint – Formulation	1,448	?	?	
4b) Lacquers and paint – Processing	1,448	?	?	
5a) Printing ink – Formulation	1,661	?	?	
5b) Printing ink – Processing	1,661	?	?	
6a) Ceramic – formulation	29	?	?	
6b) Ceramic – Processing	29	?	?	
7) Municipal STP Including emissions from private use of end products and life cycle stages 2f, 2g, and 5b.				E
8) Waste:				F
8a) Paper recycling				
8b) Car shredding				
8c) Incineration of waste				
8d) Disposal of waste on dump sites				
8e) Waste remaining in the environment				

TGD default emission factors have been used for production sites where emission data are missing. Emissions during distribution of pure DEHP (losses during transport, mainly cleaning of vessels) have been estimated in the literature. The emission from transport is not expected to cause any distinct local emissions. Its contribution is therefore added to the formulation/processing scenario in the EUSES calculation (only for calc. Regional PEC). The figures on consumption (90%ile) are assumptions based on confidential data from industry (Industry, 1999)

A: 85,680 tpa DEHP are used for the production of a semi-product (compound). The production of compound can be described in formulation followed by processing, in this case extrusion. The compound is then sold for further processing. This processing is extrusion, which stands for 48% of the used amount of DEHP in compound, injection moulding, 50% and various plastisol processes, 2%.

B: The formulation and processing scenarios are divided into two groups, polymer and non-polymer applications. This has been deemed necessary because emission patterns are expected to differ.

C: The scenario injection moulding/extrusion include 50% of the amount of DEHP in compound is used in products processed by injection moulding and 48% is processed by extrusion (**Table 3.5**; Scenario 2f)

D: The polymer-processing steps injection moulding and extrusion involve little exposure of hot product to the surrounding air and hence give rise to relatively small emissions of DEHP to the air. This is, however, not the case for other polymer-processing steps (production of sheet and

film by calendering or spread coating). In calendering large areas of flexible PVC are heated on open rollers giving rise to evaporation of plasticiser.

E: Several emissions are concentrated to municipal STPs. In this assessment emissions from private use of polymer and non-polymer products and emissions from some industrial uses (2f, 2g and 5b) is therefore added to a single local scenario. This scenario is called " Municipal STP". This combined exposure scenario makes it easier to compare calculated PEC with monitoring data (e.g. in sludge from municipal sewage treatment plants).

F: Five disposal scenarios are relevant: (i) Paper recycling, (ii) Car shredding, (iii) Incineration of end products, (iv), Disposal of end products, (v) Waste remaining in the environment. The incineration stations and dumpsites (land fills) are point sources for which local concentrations can be calculated. The releases from waste remaining in the environment are thought to be diffuse and widely disperse.

A realistic worst case estimation of emissions from incineration is based on the average incineration capacity in the EU. Emissions from dumpsites are estimated based on a national monitoring data and re-calculated to estimate emissions on an EU scale.

The different industry categories (IC), use categories (UC) and main categories (MC) used for the different life cycle stages in the assessment are summarised in **Table 2.6**.

Table 2.6 Different industry categories (IC), use categories (UC) and main categories (MC) used for the different life cycle stages in the EUSES calculations (According to TGD)

Life cycle stage	Product type	Handling type	Industry category (IC)[4]	Use category (UC)[5]	Main category (MC)	A-Table[1]	B-Table[2]
1a	Raw material	Production	2	47	Ib	A1.1	B1.3
1b		Transportation	15	55	-	-	-
2	Polymer: All products	Formulation/Processing	11	47[3]	II[3]	A3.2	B3.9
3a	Non-polymer:	Formulation	16	47	III	A2.1	B2.2
3b	Sealant, adhesives etc.	Processing/Application	16	47	II	A3.16	B3.14
4a	Non-polymer:	Formulation	14	47[7]	III	A2.1	B2.2
4b	Lacquers and paints	Processing/Application	14	47[7]	III	A3.15	B3.13
5a	Non-polymer:	Formulation	16	52	III	A2.1	B2.2
5b	Printing inks	Processing/Application	12	48	III	A3.12	B3.10[8]
6	Non-polymer: Ceramics	Formulation	16	48	III	A2.1	B2.2
7	Municipal STP	End-product uses Diffuse ind. uses	15 -	55 -	- -	-	B4.1 -
8	Waste	All waste categories	-	-	-	-	-
8a	Waste	Paper recycling			[6]	[6]	[6]
8b	Waste	Car shredder	15	55	-	-	-
8c	Waste	Incineration	15	55	-	-	B5.3
8d	Waste	Municipal land fill	15	55	-	-	B5.3

Table 2.6 continued overleaf

Table 2.6 continued Different industry categories (IC), use categories (UC) and main categories (MC) used for the different life cycle stages in the EUSES calculations (According to TGD)

Life cycle stage	Product type	Handling type	Industry category (IC)[4]	Use category (UC)[5]	Main category (MC)	A-Table[1]	B-Table[2]
8e	Waste	Waste remaining in the environment	2	47	-	-	-

1) To select emission factors.
2) To select f-value and emission days
3) Sub categories: "Polymer processing" + "Thermoplastics: plasticisers" + inclusion into or onto a matrix
4) Since this scenario is limited to emission during application, emission during end-use is added (see text).
5) 2 = chem. ind. basic chem;15=others;11=polymers ind;16=enginering ind;14=paints, lacquers and varnishes ind
6) 47 = softeners; 55 = others; 52 = viscosity adjustors; 48 = solvents.
7) Separate calculation for emission during paper recycling.
8) Sub categories: "Solvent based" + "Construction, maintenance etc" ("water based" is assumed in local scenario)
9) Sub categories: "Large company" is used.

2.6.3 Product life times and accumulation in the technosphere

Due to the high persistency of the polymer matrix in the environment, emissions from polymer end products are expected to last during a long period of time (See Section 3.1.1.3). DEHP is assumed to be persistent as long as the molecule remains in the matrix. The total lifetime of DEHP/polymer end products is therefore an important element in the estimation of the total emissions.

The contribution of emissions from waste is assumed to be high. The efficiency of waste collection and waste management strategy (recycling, incineration, landfill) will therefore have a large influence on the emissions.

2.7 TRENDS

The consumed amount of DOP (di-octyl phthalates including DEHP) has been relatively constant during the period from 1979 to 1998. See **Figure 2.3**. No data on the proportion between DEHP and DIOP (di-octyl phthalate) or the proportion between the different applications have been submitted. A consumed amount of DEHP in 1973 of 311 ktpa in seven of the present EU member states (NL, IR, UK, FR, DK, FRG, BEL) can be calculated with data from the ECDIN database.

No data from 1973 from Italy, Sweden, Finland, Austria, Spain, Portugal, Greece and Luxembourg are available. A table of the plasticiser consumption in Western Europe in 1990 gives that these countries (and Norway and Switzerland) consumed 39% of the total consumption of DEHP and DIOP in 1990 (ECPI, 1996b). From this data a total consumption of DEHP of approximately 500 ktpa in 1973 can be calculated in rough outline. An estimation from one of the manufacturers says that the production of DIOP in the 1970's was approximately 10-20% of the production of DEHP (pers. comm. Neste Oxo, 2000). If the consumption of DEHP was 500 ktpa in 1973 and the consumed amount of DIOP had the same relation to the consumed amount of DEHP as the relation of the produced amounts, the consumption of DIOP was 50-100 ktpa. The consumption of DIOP in EU, 1987, was 40 ktpa (ECDIN). The total market volume of DIOP and another phthalate DL911P (1,2-Benzenedicarboxylic acid, di-C9-11-alkyl esters, branched and linear CAS. No. 68515-43-5) in 1990 in Western Europe

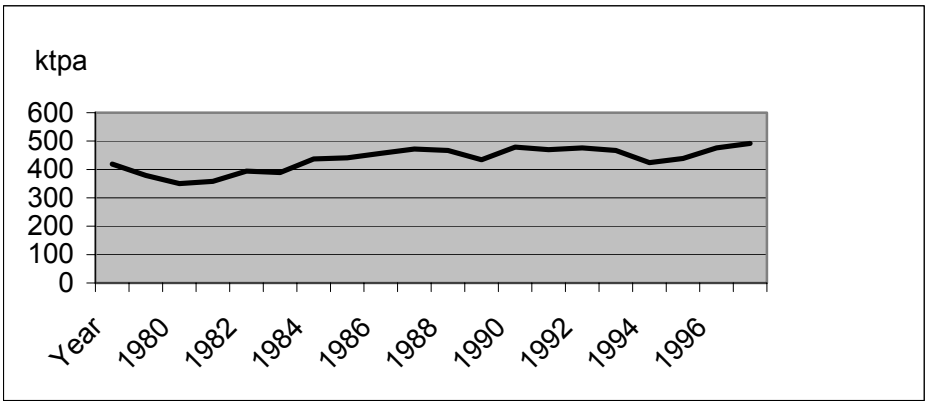
was estimated at 10-20 ktpa (ECPI, 1996b). Two producers have reported a production of DIOP of 5,000-10,000 tonnes in 1991 and 1,000-5,000 tonnes in 1993 respectively (IUCLID).

A confusing fact is that DEHP can have been named DOP (Di-octyl phthalate) or DIOP (Di-iso octyl phthalate) at some occasions, which make the retrospective description of the consumption of the substances difficult.

Taking this data into consideration it can be assumed that the consumption of DEHP has been significant the last 30 years. The consumption of DIOP was probably higher in the 70's than today.

Recent information from industry (David Cadogan, ECPI, personal communication, May 2005) shows that the use of DEHP has decreased by more than 50% from 476 ktpa 1997 to 221 ktpa 2004, whilst the use of the phthalates DINP and DIDP have increased during the same period.

Figure 2.3 Consumption of DEHP and DIOP (di-isooctylphthalate, CAS. No. 27554-26-3) in Western Europe 1979-1998. (Source: CEFIC)



2.8 LEGISLATIVE CONTROLS

Identified Occupational Exposure Limits (OELs) for DEHP are presented in **Table 2.7**.

Table 2.7 Occupational Exposure Limits for DEHP

Country	8-hour TWA (mg/m³)	STEL	Notes
Austria	5		
Denmark	3		
Sweden (HGV)	3	5 (15 minutes)	
Great Britain (OES)	5	10	
Germany (MAK)	10	100 (30 minutes)[1]	Pregnancy[3]
Netherlands (MAC)	5		
U.S.A. (ACGIH)	5	10 (15 minutes)[2]	
U.S.A. (OSHA)	5		
Czech Republic	5	10	

Table 2.7 continued overleaf

Table 2.7 continued Occupational Exposure Limits for DEHP

Country	8-hour TWA (mg/m^3)	STEL	
France (VME)	5		
Belgium	5	10	

TWA Time Weighted Average;
STEL Short Term Exposure Limit
1) Once per day.
2) Four times per day.
3) No effects on pregnancy are expected at exposure levels below MAK and BAT-values.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental releases

3.1.1.1 Release from production

Emission to air is reported from eleven of twelve sites. Measured emission data for water are available from eleven of twelve production sites as emitted amount to waste water (see **Table 3.1**) and/or concentration in the effluent (see **Table 3.37**). According to information from ten of twelve sites agricultural soil is not exposed since STP sludge from production sites is land filled or incinerated. The situation for the other two sites is unknown. Ten of twelve producers have reported emissions to urban/industrial soils. Six of these have reported zero emissions. Only site 4, 5, 7 and 8 are still producing DEHP 2006. The total EU-production of DEHP 2004 was 247 ktpa, which is only slightly more than the total production of sites 4, 5, 7 and 8 in 1998 (David Cadogan, ECPI, personal communication, March 2006). When emission data are missing a TGD default emission factor is used. For emission to air there is no default value. Instead the highest emission factor derived from emissions reported from other production sites (site 5) is used.

Table 3.1 DEHP emissions from production sites in EU 1998 (ECPI 2000) (bold type = values based on TGD defaults or highest reported).Shaded sites still in production 2006

Prod Site.	Prod. Volume	Prod. days per year	Emissions						
			To Air		To Urban Soil		To Waste Water before STP		
	tpa		tpa	Emission factor*10^{-6}	tpa	Emission factor*10^{-6}	Annual release tpa	During prod kg/d.	Emission factor*10^{-6}
Site 1	86,000	365	0.03	0.35	0	0	0.432	1.18	5.0
Site 2	77,000	340	0.03	0.39	0	0	10.6	31.2	138
Site 3	60,000	360	0	0	0	0	180	500	3,000
Site 4	58,000	365	0.025	0.43	0	0	0.0075	0.021	0.13
Site 5	57,000	351	< 1	< 17.5	0.1	1.75	171	487	3,000
Site 6	57,000	365	0.72	12.63	0.042	0.74	30.5	83.6	535
Site 7	56,000	365	0.025	0.45	5.6	100	0.0018	0.0049	0.03
Site 8	47,000	365	0.005	0.11	0.73	15.5	141	386	3,000
Site 9	41,000	365	0.01	0.24	0	0	123	337	3,000
Site 10	17,000	365	0.001	0.06	0.088	5.18	0	0	0

Table 3.1 continued overleaf

Table 3.1 continued DEHP emissions from production sites in EU 1998 (ECPI 2000) (bold type = values based on TGD defaults or highest reported).Shaded sites still in production 2006

Prod Site.	Prod. Volume	Prod. days per year	Emissions						
			To Air		To Urban Soil		To Waste Water before STP		
	tpa		tpa	Emission factor*10^{-6}	tpa	Emission factor*10^{-6}	Annual release tpa	During prod kg/d.	Emission factor*10^{-6}
Site 11	13,000	170	0.020	1.54	0	0	0.985	5.79	76
Site 12	8,000	300	0.14	17.5	0.8	100	24	80	3,000
Total*	**577,000**	**340****	**2.0**		**7.4**		**682**	**1,912**	

* Used as input in EUSES calculation (to Table 3.2)
** Average

The total emissions from production are summarised in **Table 3.2**. The continental/regional split is based on the fact that the largest production site represents 15% of the total production. These figures are used as input from production in the calculation of regional PECs.

Table 3.2 Emission from production (life cycle stage 1a)

Compartment	Total (tpa)	Continental** (85%)	Regional** (15%)
Air	2.0	1.7	0.30
Waste water*	682	579	102
Soil, urban/ind.	7.4	6.3	1.1
Total	**691**	**587**	**104**

* In the EUSES calculation 30% of these releases will be oriented directly to surface water.
** Used as input data in the EUSES model. The regional part is set to be 15% based on the production volume at the largest production site.

The release during distribution of pure DEHP ("Transportation") is addressed to the cleaning of transport vessels. It is assumed that this release is located to the waste water system outside the production site. This release is estimated by ECPI (1996a) to be 40 tpa. The estimation is based on a total "consumed" volume of 476,000 tpa. However, the release from distribution is not only restricted to the "consumed" volume. Also the imported and exported volume will be "Transported". The releases during export should therefore be included in the release estimation from the production site. The total release from distribution in the ECPI document is therefore corrected to (595,000/476,000) · 40 = 50 tpa (based on data from 1997). A local release point cannot be defined for this emission. The release is therefore used only for calculating regional/continental PECs. The emissions from "Transportation" are summarised in **Table 3.3**.

Table 3.3 Emission from "Transportation" (life cycle stage 1b)

Compartment	Total** (tpa)	Continental** (90%)	Regional** (10%)
Air	0	0	0
Waste water*	50	45	5
Soil, urban/ind.	0	0	0
Total	**50**	**45**	**5**

* In the EUSES calculation 30% of these releases will be oriented directly to surface water.
** Used as input data in the EUSES use pattern "2 prod." (see Table 3.48).

3.1.1.2 Different industrial uses (life cycle stages 2-6)

The main volume, 97-98%, of produced DEHP is used as plasticizer in polymers (mainly PVC). Estimations of emission from polymer applications are only available for PVC-polymers. In this assessment it is assumed that other non-PVC polymer applications do not diverge considerably from PVC applications in their emission pattern. In the calculations of emissions the volume for non-PVC polymer, (about 2%) is therefore added to the amount of DEHP- used for PVC-applications.

DEHP used in non-polymers is 2-3% of the total production volume. Based on a worst-case assumption that emissions from non-polymer use are higher, the highest value in the range (3%) is used. The use/functions of this volume and the emission potential are poorly known.

3.1.1.2.1 Polymer - release during industrial use (life cycle stages 2a-g)

There are approximately 800 soft PVC converting plants in EU15 relevant in the scope of this emission statement. During the spring of 2005, EuPC sent a questionnaire to 45 companies and five EuPC Sectoral Members Associations/Groups asking for information on emissions of DEHP from the conversion sites. Of those contacted, 21 companies or groups of companies answered. The companies that replied are mainly large ones, but there is also some of medium size, and are located in seven countries throughout EU15. Together they represent a volume of approximately 900 out of a total of 3,800 ktonnes (or approximately 24%) of converted products, and include all the major converting processes and most of the soft PVC applications (not cables, hoses and footwear). Thirteen sites, representing approximately 3.5% (7% if the use of DEHP has been halved since 1999) of the total DEHP use have also supplied water samples that have been analysed by IVL, the Swedish environmental research institute Ltd, and quantitative data (on flow rates etc) on their releases. Three sites supplied data on DEHP emissions to air. These were based on exhaust air monitoring. The thirteen sites use of DEHP range from 250 to over 3,000 tonnes/year, and are presented in **Table 3.4**. The quantity and quality of data recieved has been deemed relevant, reliable and adequate for the purpose of making local scenarios for the reporting sites. The information has however not been deemed adequate for extrapolation to all soft PVC converting and adhesives/sealants formulating sites. The coverage is too small, also there is a risk of (unintentional) bias in the selection of samples. For example, larger sites and sites with a focus on environmental issues are more likely to answer and also more likely to apply measures (organisational and engineering) to reduce emissions. Furthermore in order to generalize on emission levels, more background information on what are the critical parameters

for quantifying emissions are needed. The information received has therefore been used to make site-specific local risk characterisations, but the reported values have not been extrapolated to sites without reported values. For the generic sites default emission values have been used.

Table 3.4 Summary of processing sites reporting releases

Site	Process	Release to water	Release to air	DEHP usage, tonnes/year
F1	Spread coating	reported	reported	1,500
F2	Extrusion	reported	-	3,500
F3	Calendering	reported	-	800
S4	Calendering	reported	reported	1,100
S5	Compounding	reported	-	300
S6	Calendering	reported	-	1,800
F7	Extrusion	reported	-	2,000
S8	Spread coating	reported	-	250
I9	Extrusion	reported	reported	1,700
F10	Adhesives	reported	-	150
S11	Extrusion	reported	-	300
Es12	Polymer, not specified	reported	-	1,000
Es13	Polymer, not specified	reported	-	300

There has been a drastic decline in the use of DEHP from 1999 to 2004, with DINP/DIDP being the main alternatives, and several of the contacted companies no longer use DEHP. Other phthalates have and are used under similar practices and processes and with similar treatment techniques. It is therefore reasonable to assume that emissions will be similar as well.

Exhaust air treatment is used by 88% of the responding companies. Condensation, filtration and incineration are the main techniques, with one company using electrostatic precipitation in conjunction with condensation. Companies that did not use exhaust air treatment were using processes where emissions of plasticizers are likely to be lowest.

Emissions to water are mainly occasional and occur as the machinery is being cleaned. There is minimal contact with effluent water as cooling water is usually in closed loop systems. One third of the responding companies reported that there were no waste water, and 10% reported continuous emissions to waste water. In all the latter cases the water is either treated on site or sent to an external water treatment plant. There was little information on the fate of the sludge from the water treatment plant. When information was available, it indicated that the sludge was incinerated in 50% of the cases, landfilled in 20% and spread on agricultural land in 30% of the cases.

Emissions to soil were reported to be unlikely or virtually non-existent. Theoretically, accidental leakage from storage and condensation of air emissions could end up in soil.

Emission factors for PVC applications are derived from Use Category Document “Plastic Additives” (UCD draft; 1998) for regional releases and generic local sites. The reported

measured releases are used to calculate site-specific local PECs. For calculation of emissions of DEHP from polymers at formulation, processing and end-use, volumes from **Table 2.3** are used.

The techniques for formulation and processing are described in Section 2.4.2.

Based on information from the industry, PVC formulation and processing activities mainly are located at the same sites, but for a significant fraction the formulation (compounding) and the processing of articles are located at separate sites.

For one of the formulation techniques dry blending, a significant fraction is handled as a semi-product, which is processed further on other sites. A dry powder is obtained (the so-called "dry blend"), which can be stored and shipped in bulk or bags. The blended powder can be conveyed to a compounding extruder producing "compound". Further processing may occur via extrusion, injection moulding and sometimes calendering at the same or at another site "downstream". The amount of dry-blend compound is known but not the number of sites processing the compound downstream or the consumed amount of DEHP per site.

For the other formulation technique plastisol blending, data on the total amount formulated and processed at separate sites is limited. No data on the consumed amount of DEHP per site or the number of sites is available.

The combination and division of sites with formulation and processing, as it is described above, will probably influence the local and regional release patterns. Higher releases can be expected as an effect of addition of releases. This effect may be reduced by better conditions for cleaning equipment.

PVC is processed in a number of ways – calendering, extrusion, injection moulding and several plastisol applications including spread coating (with oven fusion/gelation), rotational moulding, spray coating (with closed tunnel ovens and miscellaneous small to very small applications, see Section 2.4.2.1). Emissions for each processing step have been estimated. The release factors are presented in **Table 3.5**. For the local scale, each processing technique is considered to take place in a separate factory. This is confirmed by a proprietary market analysis performed by the main producers (INDUSTRY, 1999). Local emissions due to raw material handling and formulation are included with the releases from processing.

Industry (1999) has established the consumption of DEHP at processing sites throughout Western Europe. The study covered a majority of the consumption of DEHP in Western Europe. Based on a summary statistical evaluation the 90-percentile consumption of DEHP per site for different business segments could be derived, as presented in **Table 3.5**. The data from **Table 3.5** are considered to be realistic worst case values and will be used for the derivation of C_{local}.

The life cycle stage 2e "Other plastisol" is heterogeneous because it includes car under body coating and various other plastisol processes. Except for the emissions from "Raw Materials handling" the emission from car under body coating is assumed to be insignificant. For the other plastisol processes in this scenario, (e.g. rotational coating, dip coating and slush moulding) release factors of 0.05% for sites with air cleaning and 0.5% for sites without air cleaning are suggested in the "Use Category Document for Plastic Additives".

In two life cycle stages, 2f and 2g, processing without raw materials handling or formulation is taking place.

During processing DEHP is released primarily to air. In this assessment it is assumed that 50% of the primary release will be to air. The rest is assumed to be released to wastewater, in accordance with the Emission Scenario Document (ESD) on plastics additives. In the ESD it is

assumed that releases will mainly be to air at elevated temperature, but that subsequent condensation could result in losses to liquid waste. Therefore as a worst case, 50% could be assumed to be released to air and 50% to liquid waste. The reported values for release to air are generally based on measurements at "end-of-pipe", and can therefore not be directly compared to the default values based on the ESD.

The local PECs for the different types of polymer formulation/processing sites are calculated manually except for "soil" and "worm-oral" for which EUSES 1.0 was used. (see Section 3.1.4, **Table 3.51**).

The regional contribution from polymer formulation/processing is calculated by adding the average total emissions from the different processes together. The regional release is assumed to be 10% (TGD default) of the total release (See **Table 3.6**).

Downstream processing of PVC-compound (lifecycle stages 2f and 2g) is assumed to be performed at a large number of small factories. These are assumed to be connected to municipal STPs. The wastewater releases from these life cycle stages are therefore excluded from the EUSES calculation for polymer processing and added to the local wastewater scenario for end-product use (life cycle stage 7 a) "Municipal STP" ("EUSES use pattern 1 priv.", see **Table 3.48**).

Table 3.5 Releases of DEHP from different types of polymer formulation/processing sites

Life cycle stage	Unit	2a Calendering	2b Extrusion compound	2c Extrusion product	2d Plastisol spread coating[1]	2e Other Plastisol[2]	2f[6] Injection Moulding/ Extrusion[5]	2g[6] Various plastisol Applications[3]	Total
Total EU volume	tpa	106,148	85,680	138,040	115,192	16,660	83,680	2,000	
Local releases:									
DEHP consumption per site 90.th percentile	tpa	2,824	25,00	2,500	1,500	800	?	?	
DEHP consumption per site (365 days/year)	kg/d	7,737	6,849	6,849	4,110	2,192	?		
Number of emission days/year	days	300	300	300	300	300			
DEHP consumption per emission day	kg/d	9,313	8,333	8,333	5,000	2,667			
Release - raw materials handling[4]	%	0.01	0.01	0.01	0.01	0.01	-	-	
Release – formulation[4]	%	0.01	0.01	0.01	0	0	-	-	
Release – conversion[4] *average air cleaning* *with air cleaning* *without air cleaning*	%	0.05	0.01	0.01	0.1625[1] 0.05 0.5	0.2[2] 0.05 0.5	0.01	0.5	
Release – total *average air cleaning* *with air cleaning* *without air cleaning*	%	0.07	0.03	0.03	0.1725[1] 0.06 0.51	0.21[2] 0.06 0.51	0.01	0.5	
Release per site. Average air cleaning (to Table 3.51)	kg/d	6.6	2.50	2.50	8.63	5.57	?	?	
Release per site. With air cleaning	kg/d				3.0	1.6			
Release per site. Without air cleaning	kg/d				25.5	13.6			

Table 3.5 continued overleaf

Table 3.5 continued Releases of DEHP from different types of polymer formulation/processing sites

Life cycle stage	Unit	2a Calendering	2b Extrusion compound	2c Extrusion product	2d Plastisol spread coating[1]	2e Other Plastisol[2]	2f[6] Injection Moulding/ Extrusion[5]	2g[6] Various plastisol Applications[3]	Total
Environmental distribution:									to EUSES
Total annual release per process type	tpa	74.3	25.7	41.4	198.7	35	8.4	10	Calc.[7]
Release to air (50% of tot.)	tpa	37.2	12.9	20.7	99.4	17.5	(4.2)	(5)	188
Release to STP (50% of tot.)	tpa	37.2	12.9	20.7	99.4	17.5	(4.2)	(5)	188
Release to surface water (0% of tot.)	tpa	0	0	0	0	0	0	0	0
Release to urban soil (0% of tot.)	tpa	0	0	0	0	0	0	0	0

1) 75% of all consumption is processed with air cleaning (release factor 0.05%) and 25% is processed without air cleaning (release factor 0.5%).
2) 18% is processed with air cleaning (release factor 0.05%) and 38% processed without air cleaning (release factor 0.5%). The average emission factor for the process type is then 0.2%. The emission for the process type if no air cleaning is used (emission factor=0.5%) is marked with italics.
3) 100% of all consumption is assumed to be processed without air cleaning (release factor 0.5%) because no data are available. Downstream use. Plastisol source unknown.
4) UCD draft, 1998.
5) 48% of the amount in this life cycle stage is extruded and 50% is injection moulded, both with a release factor of 0.01%.
6) The emissions from life cycle stages 2f and 2g are added to life cycle stage 7 (Table 3.28)
7) To Table 3.6.

Table 3.6 The total emissions from all polymer formulation/ processing sites to different environmental compartments. Used as input data for EUSES calculation of PECregional

Compartment	Total[2] (tpa)	Continental[1] 90%	Regional[1] 10%
Air	188	169	18.8
Waste water[3]	188	169	18.8
Soil, urban/ind.	0	0	0
Total	**375**	**338**	**38**

1) Input data in the EUSES calculation (use pattern "3 proc.")
2) From Table 3.5 ("Total")
3) In the EUSES calculation 30% of this release will be oriented directly to surface water.

Local site-specific releases to water have been calculated using measured data. Releases to air have been based on reported values where they are available (for three sites), and when reported values are lacking, they have been calculated using default emission factors from the Emission Scenario Document on Plastics Additives (OECD 2004) and reported used quantity of DEHP. For the site-specific scenarios, a very conservative approach was taken as a first step. The highest reported hourly release to water was taken for each site, and was multiplied with 24 hours/day and 300 days/year to give yearly releases for that site. For air releases, for each site, the highest value of releases from a range of years was chosen. This approach was taken as a first step because only a limited number of measured values were available (one to three per site). Where no information was available it was assumed that it was an open process, and that air treatment is used only at sites using more than 250 tonnes DEHP/year (as suggested in the ESD).

The reported site specific releases to water are presented in **Table 3.7**, the reported releases to air in **Table 3.8**, emission factors and calculated releases to air are shown in **Table 3.9**.

Table 3.7 Reported releases to water from processing sites

Site	Process	Release to surface water, (mg/h)	Note
F1	Spread coating	26	treated sewage
F2	Extrusion	306	plant discharge, 050318
		85	plant discharge, 050321
F3	Calendering	1	plant discharge, 050321
		36	plant discharge, 050322
		26	plant discharge, rain water, 050322
S4	Calendering	24	reservoir
		19	after oil-trap

Table 3.7 continued overleaf

Table 3.7 continued Reported releases to water from processing sites

Site	Process	Release to surface water, (mg/h)	Note
S5	Compounding	1	plant discharge, sample 1
		1	plant discharge, sample 2
S6	Calendering	135	effluent, 050328
		147	effluent, 050322
F7	Extrusion	27	plant discharge, 050321
		81	plant discharge, 050322
S8	Spread coating	33	plant discharge
I9	Extrusion	8	plant discharge, sample 1
		2	plant discharge, sample 2
		79	plant discharge, sample 3
		207	plant discharge, sample 4
S11	Extrusion	0	plant discharge, 050401
		0	plant discharge, 050402
Es12	Polymer, not specified	0	well source, 050405
		0	discharge, 050406
Es13	Polymer, not specified	27	untreated sewage water
		0	water for industrial use

Table 3.8 Reported releases to air from processing sites

Site	Process	Release to air, (g/h)		Year	Air treatment
F1	Spread coating	43			some incineration
		9			
S4	Calendering	101 (from calender)	-	1998	not reported
		104	18 (from extruder)	1999	
		81	12	2000	
		82	23	2001	
		95	-	2002	
		38	15	2003	
		83	28	2004	
I9	Extrusion	< 2.2 (extrusion, granulation)	< 1.6 (blending)		no air treatment

Table 3.9 Calculated site-specific releases to air from processing

Site	Process	Release to air (kg/year)	Emission factor used (%)	Description in ESD
F2	Extrusion	175	0.005	extrusion, air treatment in use
F3	Calendering	200	0.025	calendering, air treatment in use
S5	Compounding	15	0.005	comp, dry blending, air treatment in use
S6	Calendering	450	0.025	calendering, air treatment in use
F7	Extrusion	100	0.005	extrusion, air treatment in use
S8	Spread coating	1250	0.5	spread coating, no air treatment
S11	Extrusion	15	0.005	extrusion, air treatment in use
Es12	Polymer, not known	250	0.025	default, open process
Es13	Polymer, not known	75	0.025	default, open process

3.1.1.2.2 Non-polymer - release during industrial use (life cycle stage 3-6)

In Europe approximately 14,300 tonnes/annum of DEHP is consumed in non-PVC applications. Non-PVC applications include adhesives/sealant and paints/lacquers. Furthermore, DEHP is applied in non-polymer applications, such as printing inks for plastics, paper and textiles and in ceramics. Information on consumption of phthalates in non-PVC end-uses was available from a market survey from 1997 (INDUSTRY, 2000).

Table 3.10 shows the breakdown of the consumption of DEHP in the EU in non-PVC products.

Table 3.10 Annual consumed amounts of DEHP in non-polymer uses

End use	Consumption in Europe (tpa)	Fraction of tonnage For application[1]
Sealant, adhesive etc	11,142	0.023408
Lacquers and paints	1,448	0.003042
Printing inks	1,661	0.003489
Ceramics	29	0.000061
Total	**14,280**	**0.03**

1) Input data in EUSES calculations (use pattern 4-7).

According to the Swedish product register non-polymer products contain between 0.2 and 50% DEHP. Paints contain at maximum 40% DEHP.

In printing inks DEHP, is used as solvent especially for inks used in textile industry and inks used on plastics and paper.

The consumption of binders and plasticisers in ceramics in Western Europe was estimated to be approximately 5,800 tpa in 1996. Of these, the major part represents binders and of the plasticisers, the biggest share is occupied by polyalkylene glycols (SRI, 1993). According to the German Ceramics Industry Association (Hoenen, 2000) the annual consumption volume is approximately 29 tpa.

In the assessment the non-polymer industrial uses are divided in four product types, each with a separate site for formulation and processing.

1) Sealant, adhesives etc (scenario 3a/formulation, 3b/processing)
2) Paints and lacquers (scenario 4a/formulation, 4b/processing)
3) Printing ink (for paper and plastic) (scenario 5a/formulation)
4) Ceramic production (scenario 6/formulation)

Sealant, adhesives etc (life cycle stage 3a-b)

In the EUSES calculations of emissions from formulation the input values of IC=16, UC=47 and MC=III and a fraction of DEHP in formulation of 0.1 are used. These generate a fraction of main source of 1 and a number of emission days of 300. The same emission factor for emissions to waste water of 1% as for paint can be used for this product group, as the processes are similar (see below). The resulting regional and continental emissions are presented **Table 3.11**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.11 Sealant, adhesives etc: Emissions from formulation to different environmental compartments

Compartment	Emission Factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0.0025	27.5	24.8	2.8
Waste water**	0.01	111	100	11.1
Soil, urban/ind.	0.0001	1.10	0.99	0.11
Total	**-**	**140**	**126**	**14.0**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

Local site-specific releases to water have been calculated for site F10 (adhesives formulation) using measured data. Release to air have been calculated using default emission factors from the Emission Scenario Document on Plastics Additives (OECD 2004) and reported used quantity of DEHP. The estimated releases are shown in **Table 3.12**. For the site-specific scenarios, a very conservative approach was taken as a first step. The highest reported hourly release to water was taken for each site, and was multiplied with 24 hours/day and 300 days/year to give yearly releases for that site. Where no information was available it was assumed that it was an open process, and that air treatment is used only at sites using more than 250 tonnes DEHP/year (as suggested in the ESD).

Table 3.12 Local site-specific releases from site F10 Adhesives formulation

Compartment	Emission factor (EF) (%)	Release (kg/year)	Notes
Air	0.25	375	EF from ESD plastics additives; default, open process, no air treatment
Surface water	-	0 (sample 1) 0 (sample 2)	measured, plant discharge

In the EUSES calculations of emissions from processing the input values of IC=16, UC=47, MC=II and fraction of DEHP in formulation of 0.1 are used. These generate a fraction of main source of 0.5 and a number of emission days of 300. The processing will be mostly at construction sites and therefore the releases would be mainly to solid waste. The release to waste water is probably negligible. The resulting regional and continental emissions are presented in **Table 3.13**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.13 Sealant, adhesives etc: Emissions from processing to different environmental compartments

Compartment	Emission factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0.0001	1.1	1.0	0.11
Waste water**	0	0	0	0
Soil, urban/ind.	0.005	56	50.1	5.6
Total	**-**	**57**	**51**	**5.7**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

Paints and Lacquers (life cycle stage 4a-b)

In the EUSES calculations of emissions from formulation the input values of IC=14, UC=47, MC=III and fraction of DEHP in formulation of 0.05 are used. These generate a fraction of main source of 1 and a number of emission days of 300 (Table B2.2 in TGD). This agrees with the market survey made by Industry (see **Table 2.3**). In comparison with Tables 3.1 - 3.20 of the release category document IC 14 for paints, it can be estimated that 1% is released to waste water during the formulation of paints.The resulting regional and continental emissions are presented in **Table 3.14**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.14 Paints and lacquers: Emissions from formulation to different environmental compartments

Compartment	Emission Factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0.0025	3.6	3.3	0.36
Waste water**	0.01	14.5	13.0	1.4
Soil, urban/ind.	0.0001	0.14	0.13	0.014
Total	**-**	**18**	**16**	**1.8**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

In the EUSES calculations of emissions from processing the input values of IC=14, UC=47, MC=III and fraction of DEHP in formulation of 0.05 are used. These generate a fraction of main source of 0.15 and a number of emission days of 300. The volume processed at a local site can be calculated as $(1{,}448/10) \cdot 0.15 = 22$ tpa. The majority of processed paint is assumed to be solvent based. For the regional PEC calculations with EUSES the sub category "Solvent based" is therefore chosen (Table A3.15 in TGD). Since water based paints also occur (Swedish Product Register 1998-99) this is selected as a worst case assumption for the local PEC calculations. Since "water based" processing cause five times higher waste water releases compared to

"solvent based" the "local emissions to waste water during episode" in the EUSES calculation is set to be five times higher, i.e. 0.0699 · 5 = 0.35 kg/day. The resulting regional and continental emissions are presented in **Table 3.15**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.15 Paints and lacquers: Emissions from processing to different environmental compartments

Compartment	Emission Factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0	0	0	0
Waste water**	0.001	1.5	1.3	0.14
Soil, urban/ind.	0.005	7.2	6.5	0.72
Total	**-**	**8.7**	**7.8**	**0.9**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

Printing inks (life cycle stage 5a-b)

In the EUSES calculations of emissions from formulation the input values of IC=12, UC=48, MC=III and fraction of DEHP in formulation of 0.05 (KemI 2000) are used. These generate a fraction of main source of 1 and a number of emission days of 300. According to TGD as default for formulation 2% is assumed to be released to waste water. However, for the formulation of these compounds, the same emission factor for release to waste water of 1% as for paint could be used for inks, as the processes are similar. The resulting regional and continental emissions are presented in **Table 3.16**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.16 Printing ink: Emissions from formulation to different environmental compartments

Compartment	Emission Factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0.0025	4.1	3.7	0.41
Waste water	0.01**	16.4	14.9	1.66
Soil, urban/ind.	0.0001	0.16	0.15	0.016
Total		**20.7**	**18.6**	**2.1**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

The number of processors for printing inks can be expected to be large and it is assumed that the printing works are connected to municipal STPs. The release to waste water is therefore added to the local scenario "Municipal STP" (life cycle stage 7). As a simplification also the emission to air and soil are added to this life cycle stage for the contribution to regional PECs.

As no details on the nature of DEHP containing inks and their use in paper are reported the emission factors from TGD, general Table A.3.12 (IC=12, UC=48, MC=III) proposed for solvents are used. The calculated emissions are presented in **Table 3.17**.

Table 3.17 Printing ink: Emissions from processing to different environmental compartments

Compartment	Emission factor*	Total* (tpa)	Continental 90%	Regional 10%
Air	0.05	82.0	73.8	8.2
Waste water**	0.0005	0.82	0.74	0.08
Soil, urban/ind.	0.0015	2.5	2.2	0.25
Total	**-**	**85.3**	**76.8**	**8.5**

* According to TGD
** In the EUSES calculation 30% of this release will be oriented directly to surface water.
*** To Table 3.28.

Ceramics (life cycle stage 6a-b)

Plasticizers can be used as additives for ceramics to improve their processability. They work in combination with binders to give formed, unfired parts the flexibility or deformability required for subsequent handling and processing. They may also be added to spray dried or granulated powders so that the granules crush easily during pressing. Common constituent in such form liquid are polyethylene glycol, polypropylene glycol, propylene glycol and several phthalates (SRI, 1993).

The volume used for this application is low and mainly restricted to a limited number of working places.

In the EUSES calculations of emissions from formulation of the form liquid the input values of IC=16, UC=47, MC=III and fraction of DEHP in formulation of 0.05 (SRI 1993) are used. These generate a fraction of main source of 1 and a number of emission days of 300. The resulting regional and continental emissions are presented in **Table 3.18**. The local PECs are presented in Section 3.1.3 (see **Table 3.54**).

Table 3.18 Ceramics: Emissions from formulation to different environmental compartments

Compartment	Emission factor*	Total (tpa)	Continental 90%	Regional 10%
Air	0.0025	0.07	0.07	0.007
Waste water**	0.02	0.58	0.52	0.06
Soil, urban/ind.	0.0001	0.003	0.003	0.0003
Total	**-**	**0.66**	**0.59**	**0.066**

* According to TGD.
** In the EUSES calculation 30% of this release will be oriented directly to surface water.

In the processing of ceramics DEHP is expected to be totally evaporated by heating (SRI 1993). All DEHP is assumed to be released to the ambient air during a preheating phase (according to information in Miura et.al. 1993). However, according to the German Ceramics Industry Association (Hoenen 2000) outcoming gases are oxidised to CO_2 and H_2O by special post combustion. There will then be no releases from processing of ceramics.

3.1.1.3 Releases during use of end-products (life cycle stages 7a-b)

3.1.1.3.1 General considerations

Compared to industrial point sources the calculation of emissions from end-products needs a different approach. The emissions from end products[5] are in contrast to the emissions from industrial uses often extended in time. This is especially pronounced for the polymer-based products. In the polymer matrix DEHP is assumed to be resistant to biodegradation. Since the polymer has a high environmental persistency the lifetime of the polymer will influence the emission period.

For products with service lives longer than one year the estimation of the annual emission must be compensated for the fact that products produced year 1 also contribute to the emission year 2 etc. Such a product will accumulate in the technosphere until a steady state level is reached (i.e. when produced amount of the product equals the amount of the product leaving the technosphere). If the service life of the product is longer than the period for which it has been in production the amounts of the product in the technosphere will continue to increase and consequently the total emissions from these products will continue to increase. The approach in this assessment is focused on the maximum emissions when all types of emissions have reached steady state (assuming that the present use pattern is not changed). By using the equations in **Figure 3.1** (from ECPI 1996b) the annual emissions from end products are calculated. In the equation the annual emission is multiplied with the service life. This reflects the situation when the amount of DEHP in the product has reached steady state. It should be noted that the emission is assumed to be proportional to the surface area of the product and not to the content of DEHP. Furthermore, it is assumed that the release rate is constant.

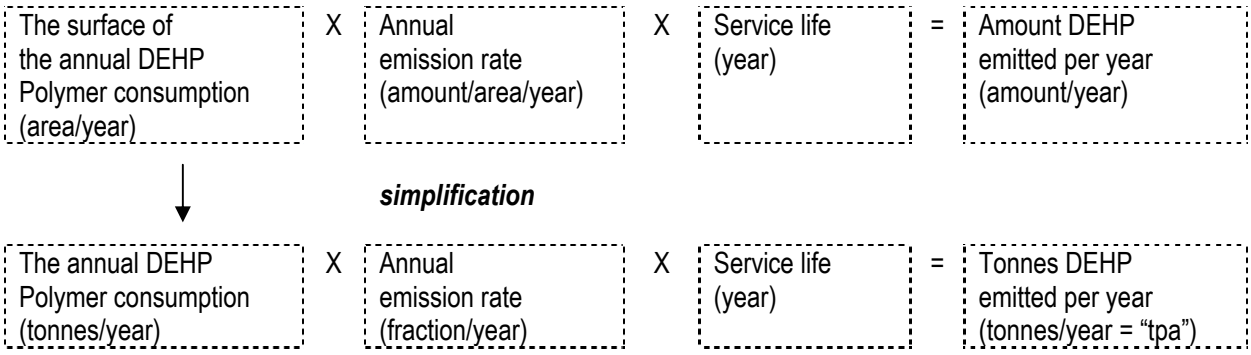
In this assessment the following definitions of different stages in a products lifetime are used:

The total lifetime:	The time period from when the product is produced until all DEHP is totally separated from the matrix. No specific release point.
The service life:	The time period from when the product (here defined as: matrix + DEHP) is produced until it ends up as waste. The release point could be the in-door or out-door environment (different emission factors and primary recipients).
The waste lifetime:	The time period from when the product is defined as waste until all DEHP has left the product matrix (mainly polymers). The release point could be the incineration station, landfills, sediment or the urban/ind. soil (different emission factors and primary recipients).

A rough estimate of the maximum total lifetime could be based on the average emission rate from a polymer product. An assumed total emission rate of 1% per year (during use, incineration and disposal) (Berntsson 1984) gives a half-life of 69 years ($\ln 2/0.01$ year^{-1}). If half of the content of DEHP in the product is released after 69 years, the total product lifetime could be expected to be more than 100 years (longer for thicker goods and shorter for thinner goods).

[5] DEHP is here defined to be emitted when it reach the surface of the polymer. The dissipation from the surface is here considered as a part of ”environmental fate” (incl. degradation and distribution).

Figure 3.1 Calculation of emissions from articles during service life



3.1.1.3.2 Polymer - Emissions during use of end-products

Three main emission routes are identified:
1) in-door use of products with emission to air
2) in-door use of products with emission to waste water
3) out-door use of products with emissions to air, water and soil

In-door use - emissions to air

The majority of flexible PVC is used indoors in applications such as flooring, wall covering, upholstery, wire and cable etc. The total volume for in-door use of DEHP in polymer products is approximately 362,000 tpa.

The evaporated amount has been estimated in two ways.

Method 1: Using a general emission factor of 0.05%/year (emission factor based on the total annual loss of the annual consumption) (UCD draft, 1998) the total emission from in-door polymer products to air would amount to: $0.05/100 \cdot 362{,}000 = 181$ tpa.

Method 2: If the surface area of the products is known, an emission factor of 9.5 mg/m^2/year can be used (Environ Corporation, 1988).

The total production of PVC-flooring in Europe is $2.3 \cdot 10^8$ m^2 (ECPI, 1998b). With a service life of 20 years the total area will be $4.6 \cdot 10^9$ m^2. 51% of the plasticisers are assumed to be DEHP. This gives $2.35 \cdot 10^9$ m^2. The total emission of DEHP from PVC-flooring to air in Europe can be calculated to: $9.5 \cdot 2.35 \cdot 10^9 = 2.23 \cdot 10^{10}$ mg/year = 22.3 tpa

Vinyl wall covering is a typical product in this group for which statistics are available. The total production in Western Europe in 1988 is estimated to be approximately $400 \cdot 10^6$ m^2 (ECPI, 1996b). 51% of the plasticisers are assumed to be DEHP. Assuming an average life-time for this application of 7 years and applying the same emission factor as for flooring gives a total emission of: $7 \cdot 400 \cdot 10^6 \cdot 0.51 \cdot 9.5 = 1.36 \cdot 10^{10}$ mg/year = 13.6 tpa.

Western European statistics for coated products, film and sheet, such as upholstery, packaging, stationary products, luggage, clothing etc. are not available. An assumption made by ECPI (ECPI, 1996b) states that a worst case estimate for the emission of phthalates from these products is 40 tpa (assuming a product lifetime of 7 years). With 51% of total phthalate consumption being DEHP the DEHP emission should be $0.51 \cdot 40 = 20$ tpa. In the ECPI document the phthalate emissions from cables is assumed to be in the magnitude of 50 tpa. With

51% of total phthalate consumption assumed to be DEHP the DEHP emission should be $0.51 \cdot 50 = 25$ tpa.

Assuming that the hose and profile (10 years service life, DEHP consumption 57,120 tpa) has a similar surface to volume ratio as flooring (DEHP consumption 73,780 tpa emitting 22.3 tpa). The DEHP emission from these products is calculated to be: $57{,}120/73{,}780 \cdot 22.3 \cdot 20/10 = 8.5$ tpa.

According to the information given above, the total emission of DEHP to air from in-door applications: 22.3 + 13.6 + 20 + 25 + 8.5 = 89.4 tpa

Conclusion

The more detailed method 2 should be preferred. However, the data on the different in-door uses is not detailed enough to calculate the emission from each product group based on surface areas (i.e. coated products, film and sheet). Method 1 is therefore preferred as a realistic worst case scenario. Thus, 181 tpa of DEHP is expected to evaporate from in-door use of products containing DEHP (see **Table 3.28**).

In-door use - emissions to water

Leaching of DEHP by washing clothes with PVC printing - emissions to waste water

The annual tonnage for release from washing of clothes with PVC printing is based on a Danish experimental study on T-shirts (4 different clothes, Miljöstyrelsen 1996). The release of phthalates in Denmark was estimated to be 6.9 tpa (range: 1.3 - 13). The range in the estimation indicates that the variation between different clothes is large. Based on separate analysis on the different phthalates the DEHP part can be estimated to be about 21%, i.e. 1.5 tpa (range: 0.27-2.7) tpa. With an assumption that the Danish use of PVC printing clothes is applicable to the rest of EU (realistic worst case) the total EU emission will be:

(370,000,000 inhabitants in EU)/(5,300,000 inhabitants in DK) · 1.50 tpa = 105 tpa (range: 18-180).

Leaching of DEHP by washing polymer floors - emissions to waste water

The release from washing of polymer floors is based on a Swedish experimental study (Forshaga 1996). A release of 5 $\mu g/dm^2$ per cleaning was measured (10 days cleaning interval). It should be noted that the experimental design only cover emission by diffusion (emission due to abrasion is not included). The total area for PVC-flooring is estimated to be $2.3 \cdot 10^9$ m^2 (ECPI 1998b). This is based on an assumed service life of 10 year. However, according to a producer (TARKETT 1999) 20 years is a more realistic service life. This gives a doubling of the area to $4.6 \cdot 10^9$. About 51% of this area is assumed to contain DEHP, i.e. $2.35 \cdot 10^9$ m^2. One cleaning per week is assumed. This will give:

$$2.35 \cdot 10^9 \cdot 52 \cdot 5 = 61.1\text{tpa}$$

The release caused by abrasion of the floor surface is expected to be extensive in places as offices and schools. The abrasion can be estimated by assuming a total loss during the whole service life. According to standard test for abrasion about 0.1-0.15 mm (average 0.125) will be lost during the service life (TARKETT 1999). With a thickness of 2 mm (TARKETT 1999) this will give 6.25% per 20 years or 0.312%/year. This abrasion is expected to occur on surfaces exposed to frequent walk here assumed to be half of the area. Based on these assumptions

2,302 tpa (0.5 · 20 · 73,780 · [0.312/100]) will be released as small particles. The distribution of this is unknown. Wastewater (wet cleaning) and landfills (via e.g. dry cleaner) are realistic recipients. Before cleaning some of the particles may be distributed into the air as an object for human exposure. Assuming 50% loss to waste water will give 1,151 tpa (0.5 · 2302).

The total emissions from washing and abrasion of floors will then be 61.1 + 1,151 = 1,212 tpa.

It can be questioned to add DEHP emitted in PVC-particles to the leached amount. However, the particle size is expected to be very small and the particles are here assumed to behave in the same way as pure DEHP.

The total emission of DEHP to waste water from in-door applications is calculated to be:

105 + 1,212 = 1,316 tpa (see **Table 3.28**)

Out-door use

Different products have different service lifetimes. It is therefore necessary to split the out-door uses into different groups. The following end products are identified as important sources for emissions during use:

1) Car undercoating
2) Roofing material
3) Coils coating
4) Fabric coating
5) Cables and Wires
6) Hoses and Profiles
7) Shoe soles

In the UCD draft (1998) general emissions factors for out-door uses are recommended. These are, however, to general to fit the calculations needed for DEHP. Firstly they are not considering the influence of volume/area relationship on the emission rate. Secondly, the leaching rate is not based on a study on leaching of DEHP. Thirdly, the leaching rate in the draft UCD is not correctly derived from the study. Some recalculations of the leaching rate are therefore needed.

Recalculations

The annual emissions by leaching in out-door use is estimated from studies on roofing material made by Pastuska et al. 1988 and Pastuska et.al 1990. In open-air exposure 0.16%/year was released. For surface covered with gravel 0.35%/year was released (the authors show that dirt strongly increase the emission rate). These figures are derived from PVC with a phthalate mix that contains normal C8-C10 and iso-C10 (no more details in the original report). Assuming a 50:50% mix will cause an average chain length of 9.5 compared to 8 for DEHP. Normally the migration rates increase with decreasing carbon length. The emission factor for DEHP is therefore expected to be higher. A comparing study on evaporation rates for DEHP and DIDP (Wilson et.al 1978) is available. Here the evaporation rate at 40°C (7 km/hour air stream) for DEHP was estimated to be 8.5 times higher than for DIDP (0.017 compared to 0.002 g/m^2/day). This indicates that also the leaching rate may be higher for DEHP compared to DIDP. Until better data are available a doubling of the emission rate derived by Pastuska is assumed to compensate for this difference. Thus, emission rates of 0.3 and 0.7%/year for open air and gravelled roofing materials respectively will be used in the assessment.

Since the emission from a material is assumed to depend on the surface area and not on its content of DEHP the emission rate/area is calculated from the study on roofing material:

The volume of 1 m^2 roofing material (thickness 1.5 mm) is 1,000 · 1,000 · 1.5 = 1.5 · 10^6 mm^3. With a density of 1.406 mg/mm^3 (65%) for PVC (ref. Sax) and 0.985 mg/mm^3 for DEHP (35%) this correspond to [(0.65 · 1.406)+(0.35 · 0.985)] · 1.5 · 10^6= 1.89 · 10^6 mg = 1,890 g. With a starting concentration of 35 weight% DEHP and a reduction of 0.3 weight%/year for the open-air exposure the annual emission rate will be 0.3/100 · 35/100 · 1,890 = 1.98 g/m^2 (emission only from one side). The annual emission rate for gravelled surface will then be 4.62 g/m^2.

Field studies are also available for the underground use of cable and wires (de Coste, 1968 /Study I and de Coste, 1972/Study II). The techniques used and locations are almost identical in the two studies. 0.76 mm sheet samples were buried in soil in Georgia, US (humid climate, pH 5.2) at two different depths mounted on polyethene tubes. After 32 or 48 months the concentration of DEHP in the exposed PVC sheets were measured. The following decreases in concentration were observed:

Table 3.19 % DEHP loss from cable material after 32 and 48 months in soil

		Study I			**Study II**	
Exposure duration		48 month			32 month	
Original conc. DEHP		30.6%			37.2%	
Soil depth	15 cm	45 cm	average	25 cm	60 cm	average
Total % DEHP loss	4.7	3.1	3.9	0.90	2.50	1.70
% loss / year	1.18	0.78	0.98	0.34	0.94	0.64

These studies did not show any clear correlation between emission rate and depth. The observed variation may depend on uncertainty in the chemical analysis. However, it may also depend on heterogeneity in the soil compartment. The relatively low pH (5.2) in the studied soil should be considered. An identical study was also carried out in another region (Study I, New Mexico) with higher pH (8.2). The dissipation of phthalates was not measured in this study. However, the change in mechanical properties (tensile stress) of the plastics where measured. Since elongation for this type of materials is sensitive to small changes in plasticizer concentration (according to the author) this parameter can be used as an indication of phthalate dissipation. By comparing the changes in elongation it clearly indicates that the phthalate dissipation was higher in the basic soil. The levels of dissipation of DEHP in **Table 3.19** may therefore be an underestimation compared to neutral and basic soils. Due to these uncertainties the highest estimated emission rate in **Table 3.19** i.e. 1.2%, is assumed in this RAR (worst case assumption). The primary recipient is assumed to be 100% to the soil environment.

There are indications that the losses of DEHP from gravelled roofing material and soil buried cables at least in part are due to degradation at or near the surface of the polymer meaning that the lossess seen may not be an emission in the true sense. Besides the two studies cited above there are several studies available investigating plasticiser dissipation:

- Meriowsky et.al. 1999 study the long-term behaviour of PVC materials, incl. DEHP, in land fill environments (simulation tests and field studies). The result shows that DEHP can dissipate from the PVC matrix. The dissipation is considerable higher during the methanogenic (anaerobic) phase compared to the acidogenic phase. The low concentrations of DEHP in leakage water also indicate that DEHP is degraded and/or translocated within the landfill. The mechanism for dissipation is described as diffusion where an important

driving force is the removal of plasticizer from the surface of the PVC e.g. by leaching (elution and rinsing) or biodegradation. The authors suggest that the diffusion flux primarily depends on "the microbial consumption".

- Plate 1997 study degradation of DEHP in PVC film under land fill conditions. In difference to Meriowsky et al. (1999) the test where conducted in soil ("ED 73 Standard soil"). The temperature was 50°C and artificial seepage water representing "young" (pH 4.5), "old" land fills (pH 8.5) and rain water (distilled water) where percolated through the samples during 1.5 years. A control sample also was incubated at room temperature without seepage water. The results show a decrease in DEHP concentration in PVC in all tests. Highest loss was observed for "young" seepage water and the room temperature control. The high loss in the control was explained with god condition for growth at which DEHP was used as a source for nutrition. Further, the concentration of DEHP in the surrounding soils did not increase considerably. The author assumed that most of emitted DEHP was degraded or hydrolysed.
- Bessemes 1988 (Akzo Chemie America) found in a laboratory study that the bio film grows thicker if the phthalate in the PVC was easy degradable. Among several plasticizers DEHP was categorized to be in medium class for attack:

Difficult to attack:	DINP
	DHP (dihexylphthalate)
	DIOP
Moderate to attack:	DEHP
	DBP
	TOF (tri-2-ethylhexyl phosphate)
Easy to attack:	DOA (di-2-2ethylhexyl adipate)
	ESO (epoxidized soybean oil)

The author stated that the bio film is established because the plasticizer is used as a carbon source. Therefore he suggests that biocides should be added to soft PVC to prevent degradation of the plasticiser.

- The effect of a biocide on "plasticizer" loss was studied by "Morton International Brussels" (cited in ECPI 2000b). After 12 months burial in soil the biocide (conc. 1%) reduces the "plasticizer" loss from PVC about 50% (see **Table 3.20** below).

Table 3.20 Plasticiser loss after 3, 6 and 12 months of burial

% weight loss during soil burial			
Biocide added[1]	Soil burial		
	3 months	6 months	12 months
0%	0.272	0.711	1.03
0.5%	0.042	0.327	0.63
1%	0.0	0.170	0.48

1) VinyzeneR SB1 (containing 5% active material: 10:10 oxybisphenoxarsine)

- Webb et.al 1999 study the mechanism for the adhesion of a deterious fungus on PVC. He found that the phthalate seemed to change the electrostatic forces on the PVC surface in a way that stimulate fungus adhesion. Adding a respiration inhibitor to the test system did not

change the increased level of adhesion. The author therefore stated that the use of the plasticiser as a carbon source was unlikely to contribute to the observed adhesion.

- In several long term studies Pastuska (et.al 1988, et.al 1990) estimated the loss of plasticizers from PVC roofing materials during different environmental conditions. The highest loss of plasticizer was observed under exposure where organic material was present (dirt from gravel, fungi, carbon black), whereas exposure with only inorganic material had the lowest loss rate (see **Table 3.21** below). Carbon black was the most effective reducer of plasticizers. The results show that microorganisms may contribute to some of the observed losses but that moist organic dirt alone enhances the loss of plasticizers. The author therefore stated that "the application of biocides may be useful to prevent the activity of micro organisms but it cannot prevent the negative effects of organic dirt".

Table 3.21 % Dissipation of phthalates from roofing material after 1 year[1] (review of several studies)

Uncovered	Covered by gravel[2]	Dirt from gravel	Water	Carbon Black	Fungi	Fungi and nutrient	Silica gel	Silica gur
0.16	0.35	0.5	0.3	1	0.4	0.43	0.1	0.05

1) For studies lasting shorter than 1 year the loss is extrapolated.
2) As a protection roofing sheets can be covered by gravel.

From the studies above it can be concluded that microbial growth on plasticised PVC is possible and that DEHP can be utilised by microorganisms at the surface of PVC or in the surrounding environment (e.g. soil). However, in none of these studies has the degradation been quantified. Neither has it been shown that the degradation actually takes place at the surface of the polymer. The increased dissipation caused by organic dirt may to a large extent be an abiotic effect caused by increased diffusion as indicated by the study with Carbon black (Pastuska, 1988). A hypothesis is that organic dirt may act as a lipophilic diffusion bridge between PVC and the surrounding environment. In such a case the dissipation will not be caused by biodegradation. The further fate will depend on the actual conditions in the surrounding environment Therefore, a worst-case assumption is used in this assessment i.e. the observed loss of DEHP from PVC is assumed to be a release to the surrounding environment.

Used emission factors:

Air: In the UCD draft (1998) 0.05% is assumed to be evaporated for out-door use products during service life. There is no information on the material thickness and service life lying behind this value. It is therefore not possible to recalculate this figure to emission/area. Instead the emission rate for in-door use, 9.5 mg/m^2/year (see estimations of indoor emissions above) is used

Water and soil: The annual emission rate derived from roofing material, 1.98 g/m^2 for open air exposure and for gravelled surface, 4.62 g/m^2, will be used for all products except for soil buried cable (air emission excluded). The emission rate for "open air" is used for all product types except for shoe soles for which the rate from "dirty area" is used. According to UCD draft (1998) the leached part is assumed to be equally distributed to soil and water.

Soil buried cable and wires: The annual emission rate estimated from field studies, 1.2%/year is used.

Summary:

Air:	-	0.0095 g/m^2/year
Water:	open air:	0.985 g/m^2/year
	dirty area (gravelled):	2.31 g/m^2/year
Soil:	open air:	0.985 g/m^2/year
	dirty area (gravelled):	2.31 g/m^2/year
	Cable and wires:	1.2 %/year

The leaching rates from PVC-coated roofing material have been estimated by the recalculation presented above. The emissions from the other product groups can be calculated by applying a "surface correction factor" (SCF), which is defined as the relative change in surface area compared to the roofing material. In **Table 3.22** SCF for the different products are estimated.

The emissions from car undercoating are due to evaporation and leaching. Only the releases due to leaching are taken into account to estimate the releases to water. Vikelsøe et al. (1998) measured the releases of DEHP from cars to wash water in car wash centres. Phthalate concentrations were determined in wash water from two car wash stations in Denmark in 1996 and 1997. The samples were taken at the car wash station in the well collecting the washing water in the washing room. DEHP was analysed in 26 samples. The concentrations varied from 11 to 140 µg/l.

The corresponding emissions per single wash varied from < 0.5 to 110 mg/wash (mean 16 mg/wash). The concentrations varied from < 3 to 760 µg/l (mean: 112 µg/l). Assuming approximately $120 \cdot 10^6$ cars in Western Europe and two car washes per month and an average release per wash of 16 mg, the release would amount to 46 tpa distributed to waste water. Further release would also occur during normal use e.g. driving on wet roads etc. The releases during car washing can nevertheless be considered as worst case conditions, so that it can be assumed that the additional releases do not exceed those during car washing. This additional release is here assumed to be equal to the amount released from washing i.e.46 tpa equally distributed to soil and surface water.

The emission to air from car under body coating is estimated by using the emission rate for indoor use applied to the total surface area of car undercoating. The same thickness as for roofing material is assumed i.e. 1.5 mm. This gives an SCF of 1.

No data are available for emissions from coil coated sheets. Coil coating is about 10 times thinner than roofing material (0.1-0.18 mm compared to 1.5 mm, Meki 1999). This gives a SCF of 10.

No data are available for emissions from PVC-coated fabric material. However, this material is assumed to be similar to roofing material except that emissions will be possible from two sides. This will give a SCF of 2.

No data are available for emissions from hoses and profiles. However, this material is assumed to be twice as thick as the roofing material, consequently the emissions are reduced to the half. This will give a SCF of 0.5 (1.5/3).

Emissions from cables and wires are expected as evaporation and leaching. The emission rate and recipient are assumed to be different for under-ground use compared to over-ground use. About 20% are estimated to be used over-ground and 80% to be used under ground (ECPI 1999). For both over and under ground use the same thickness as for roofing material is assumed. This will give a SCF of 1. For soil buried cable and wires all emission is expected to be to the soil compartment. The contribution from soil buried cables is special. Since this release is occurring

deep in the ground it cannot be added together with the other more surface oriented releases. In TGD the urban/industrial soil depth is only 5 cm. The PEC calculations are therefore not able to cover this release. Developments of new distribution models are therefore needed (with focus on groundwater exposure). Until better models are available the contribution from soil buried cables are excluded from the PEC calculations.

No data are available for emissions from shoe soles. However, this material is assumed to be similar to the roofing material except that it is 10 times thicker. This will give an SCF of 0.15 (1.5/10).

Table 3.22 Surface Correction factor (SCF) for different polymer end products for out-door uses

Product group	Average thickness (mm)	Emission sides[1]	SCF
1. Car undercoating	1.5	single	1
2. Roofing material	1.5	single	1
3. Coil coating	0.15	single	10
4. Fabric coating	1.5	double	2
5a. Cable and wires – air	1.5	single	1
5b. Cable and wires – soil	1.5	single	1
6. Hoses and profiles	3	single	0.5
7. Shoe soles	10	single	0.15

1) Polymers applied on a non-diffusible material will only release DEHP from one side.

Volumes and service life for these groups are presented in **Table 3.23** (source: INDUSTRY, 1999).

Table 3.23 Consumed volumes, service life and resulting surface area of main groups of out-door use types (polymer end products)

Type	Volumes (tpa)	Service life (year)	Total emitting surface area[1] (m^2)
car undercoating	7,000	12	44,444,444
roofing material	1,000	20	10,582,011
coil coating	5,000	10	264,550,265
fabric coating	21,000	10	222,222,222
cable and wires - air	4,000	30	63,492,063
(cable and wires – soil[2]	16,000	30	253,968,254
hoses and profiles	6,000	10	15,873,016
shoe soles	40,000	5	15,873,016

1) [DEHP volume of the end product] · [the area of 1 tonne roofing material=529m^2] · SCF · service life
2) The surface area of soil buried cable is not used in the calculations of emissions.

The releases to different environmental compartments from out-door uses are summarised in **Table 3.24**. The result shows that on average 1.3% of DEHP is lost during the service life (soil buried cable excluded). The variation between product types lies between 0.2 and 36%.

Table 3.24 Summary of total releases from outdoor use of polymer end products

Scenario	Emission to (tpa):				Total	% of initial
	air	Surf. water	waste water	urban soil	Other soil[2]		Content
car undercoating	0.42	23	46	23	0	92.4	1
roofing material	0.10	24.4	0	24.4	0	49	5
coil coating	2.5	261	0	261	0	524	10
fabric coating	2.1	219	0	219	0	440	2
cable and wires - air	0.60	62.6	0	62.6	0	126	3
cable and wires - soil	0	0	0	0	5,760	5,760	36
hoses and profiles	0.15	15.6	0	15.6	0	31	0.5
shoe soles	0.15	36.6	0	36.6	0	73	0.2
Total[1]	**6**	**642**	**46**	**642**	**5,760**	**1,336[3]**	**1.3**

1) To Table 3.28.
2) Soil buried cables.
3) Emission from buried cable excluded

3.1.1.3.3 Non-polymer - Emissions during use of end-products

There is only limited information available on this product group. The use of DEHP in non-polymer applications is stated to be maximum 3% of the total DEHP consumption corresponding to 14,280 tpa (see **Table 2.2**). The only available information on emissions from non-polymer products is a report from US-EPA (Perwak and Slimak 1981). The authors stated that the release of phthalates during use is 80% per year with a maximum one-year product lifetime. The primary recipients are assumed to be air (40%), waste water (40%) and soil (20%). The authors made a reservation that these figures are highly uncertain. Since these figures also cover other more water-soluble phthalates (such as DBP) they probably overestimate the total release of DEHP.

Until more specific emission rates are available the emission factors derived for polymer end products are used.

As for polymer products a surface correction factor is used to estimate the emitting area of the products.

1) Sealant, adhesives etc

There is no information available on the volumes used indoors and outdoors. For the purpose of this risk assessment half of the volume is assumed to be used indoors. Since such material can be expected to be in contact with water the emission factor for leakage during outdoor use is used (1.98 g/m^2/year). As for polymers the outdoor emissions are assumed to be equally distributed to water and soil. For the indoor part, however, all emissions are assumed to be directed to waste water. The thickness of sealant, adhesives etc. are assumed to be the same as for roofing material (1.5 mm). This will give a SCF of 1 (see **Table 3.25**).

2) Lacquers and Paints

There is no information available on the volumes used indoors and outdoors. However, the same release pattern is assumed as for sealant, adhesives etc. (see above). The normal thickness of lacquers and paints is 0.04 mm (Fristad 2000). This will give a SCF of 38 (see **Table 3.25**).

3) Printing inks

All printing ink uses are assumed to be conducted indoors. As a simplification all printing inks are assumed to be used on paper. There is no information about the thickness of printing inks. Here it is assumed to be 0.0015 mm. This will give a SCF of 1,000 (see **Table 3.25**). Only emission to air is assumed to occur (emission factor 0.0095 g/m^2/year). Most of the DEHP added to paper via the ink is assumed to remain in the recycled paper (see Section 3.1.1.4). With 50% paper recycling and an assumption that 94% of the DEHP is remaining in the recycled paper the total amount of DEHP in paper is assumed to be higher than the amount added with inks. A correction factor of 2 will be used to take account of this "retention". Thus, the tonnage will be 2 · 1,661=3,322 tpa (see **Table 3.26**). The service life for printed paper is assumed to be 1 year (this is assumed to also cover the recycling steps).

Table 3.25 Surface Correction factor (SCF) for different non-polymer end products

Product type	Average thickness (mm)	Emission sides[1]	SCF
Sealant, adhesives etc.	1.5	single	1
Lacquers and paints[3]	0.04	single	38
Printing ink[2]	0.0015	single	1,000

1) Non-polymers applied on a non-diffusible material will only releases DEHP from one side.
2) No reference.
3) Average thickness according to Fristad (2000).

Table 3.26 Consumed volume, service life and resulting surface area of the main groups of non-polymer end products

Product type	Volumes (tpa)	Service life (year)[2]	Total emitting surface area[1] (m^2)
Sealant, adhesives etc	11,142	20	117,904,762
Lacquers and paint	1,448	7	201,111,111
Printing ink	3,322[3]	1	878,835,979

1) [DEHP volume of the end product] · [the area of 1 tonnes roofing material=529m2] · SCF · service life
2) From Table 2.4
3) Yearly consumption corrected for paper recycling (1,661 · 2)

The releases to different environmental compartments from out-door uses are summarised in **Table 3.27**.

Table 3.27 Summary of total releases from non-polymer end-uses

Product type		Emission to (tpa): air	surf.water	waste water	urban soil	Total
Sealants, adhesives etc	in-door	0.56	0	116	0	117
	out-door	0.56	58.1	0	58.1	117
Lacquers and paint	in-door	0.96	0	198	0	199
	out-door	0.96	99.1	0	99.1	199
Printing ink	in-door	16.7	0	0	0	16.7
Total[1]		**19.7**	**157**	**314**	**157**	**648**

1) To Table 3.28

"Municipal STP" ←→ "Diffuse emissions" (life cycle stage 7a-b, → EUSES calculations)

In the society the use of end products is mainly a source for diffuse emissions. Creating a local scenario for each type of product type is therefore not relevant. However, some of these emissions are concentrated in the municipal STP system and cause a local emission at the STP recipient. Here the in-door emissions to waste water are the main source. But also some emissions from out-door uses can be expected to reach the STP (via storm water distribution to the STP). However, in this assessment as a worst case assumption all storm water releases are oriented to surface water. Small industrial sites like printing works or smaller factories working with plastisol dip coating are assumed to be connected to municipal STPs. The wastewater releases from these life cycle stages (2f, 2g and 5b) are therefore added to the local wastewater scenario for end product use.

Estimated emissions to air, soil and surface water from polymer- and non-polymer- end products use, and small scale industrial use (life cycle stages 2f, 2g and 5b) are only used for their contribution to the calculation of continental/regional PECs (use pattern 2 priv. in the EUSES calculation).

Table 3.28 sums up all emissions from life cycle stages, 2f, 2g, 5b and 7.

Table 3.28 Total emissions from End-products uses (life cycle stage 7) and small scale industrial uses (life cycle stages, 2f, 2g and, 5b)

Compartment	Non-polymer[1] outdoor	indoor	Polymer outdoor[2]	Indoor	Small scale industrial uses 2f[3]	2g[3]	5b[4]	Total[5]
Air	1.5	18.2	6	181	4.2	5	82	299
Waste water	0	314	46	1,316	4.2	5	0.83	1,686
Surface water	157	0	642	0	0	0	0	799
Urban/Industrial soil	157	0	642	0	0	0	2.5	801
Other soil	0	0	5,760	0	0	0	0	(5,760)
Total	**316**	**333**	**7,096**	**1,497**	**8.4**	**10**	**85.3**	**3,585**

1) From Table 3.27.
2) From Table 3.24. The contribution to waste water comes from car washing.
3) From Table 3.5
4) From Table 3.17
5) To Table 3.29. Soil buried cable ("other soil") is excluded.

The continental and regional emissions are set to 90% and 10% respectively (default) and are presented in **Table 3.29**. These values are used as input data in the EUSES calculation. The releases to waste water are used to calculate a local PEC for municipal STP (see **Table 3.29**, use pattern 1 priv.). In the EUSES calculation the default value on the fraction of main source of 0.002 from B-table 4.1 in the TGD is used. The number of days for emission is set to 365 days. The remaining releases (to air, surface water and urban soil) are only used for their contribution to the regional concentrations (see **Table 1.1**, **Table 3.48**, use pattern 2 priv.).

Table 3.29 The total, regional and continental EU emissions from end-products (life cycle stage 7) uses and small-scale industrial uses (life stages 2f, 2g and 5b). The waste water emission is used for a local STP scenario (use pattern 1 priv. in EUSES)

Compartment	Total (tpa)	Continental 90%	Regional 10%
Air	299	269***	30***
Waste water*	1,686	1,518**	169**
Surface water	799	719***	80***
Soil, urban/ind.	801	721***	80***
Total	**3,585**	**3,227**	**359**

* 70% is assumed to be treated in STP and 30% be released directly to surface water (according to TGD).
** Input data to use pattern "1 priv." in EUSES
*** Input data to use pattern "2 priv." in EUSES

3.1.1.4 Release from disposal

In cases where a disposed product is not incinerated or recycled the emission period will be longer due to a longer total lifetime. The emission during the waste lifetime is in many countries mainly located to landfills. Emissions from landfills are mainly due to leaching and are technically controllable.

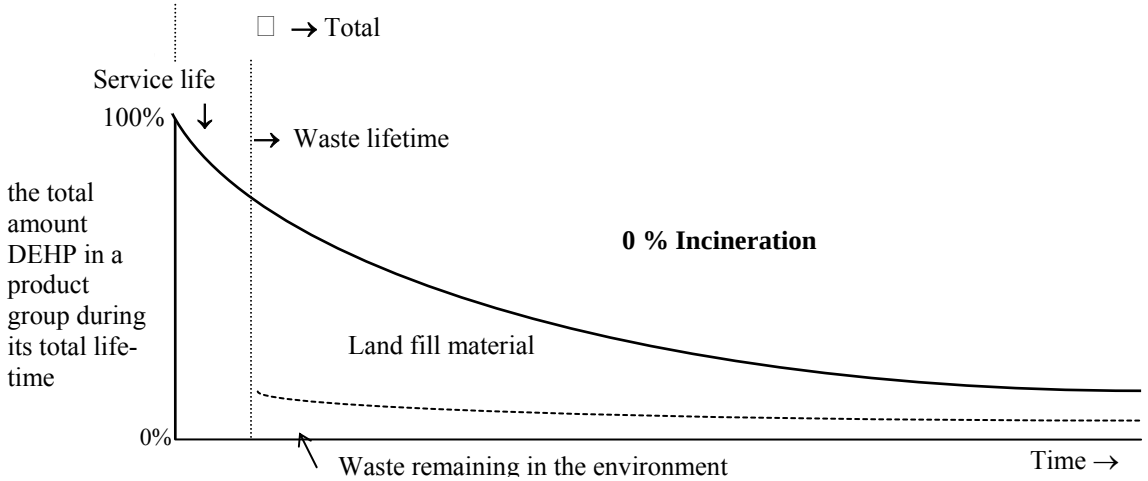
An unknown amount of polymer products will, however, end up elsewhere. Examples of such waste streams are loss of particles from car undercoating and other polymer products (caused by weathering effects). This emission is wide disperse and out of waste management control. Such waste remaining in the environment may therefore be an important contributor to the overall environmental exposure.

Figure 3.2 gives a schematic view of the amount of DEHP in a product group over its total lifetime. The different areas in the graph are roughly proportional to their contribution to the total lifetime release from polymers. It should be noted that the relatively large area representing "land fill" is an internal release from polymers within the landfill. Measured data show that the releases from the municipal landfills are much lower (due to adsorption/degradation).

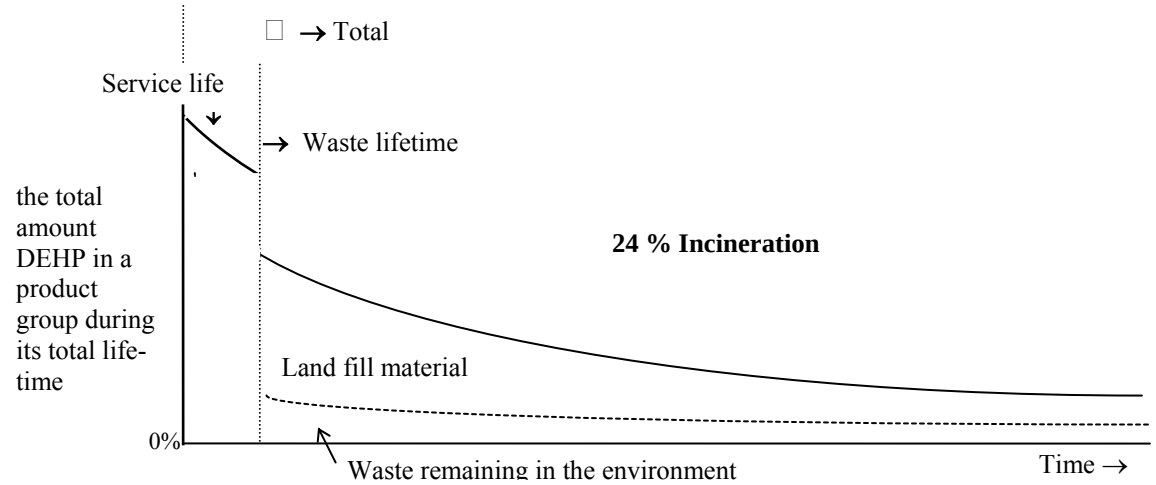
The strategies in waste management vary considerable between different EU countries (**Figure 3.2**). The incineration rate of municipal waste varies between 0 and 75% (ETSU 1996). Consequently, the rate for municipal landfill application varies between 25 and 100%.

Figure 3.2 Schematic view of the amount of DEHP in a product group from its introduction on the market until the end of its "total life-time". The thick line shows the total amount of DEHP remaining in the product group (incineration ref. ETSU 1996)

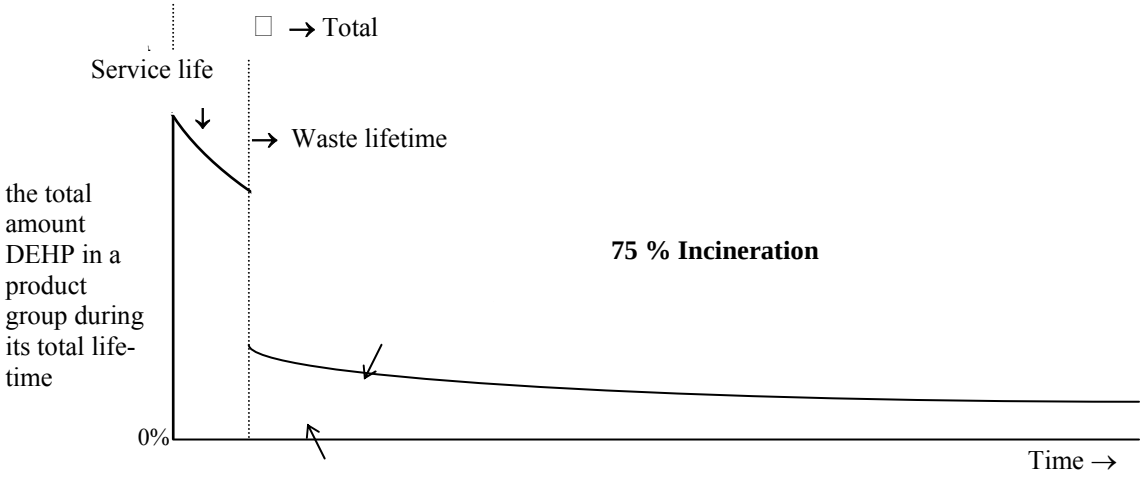
a) Only land fill (Greece, Ireland, Portugal)



b) Average in EU



c) High incineration rate (Luxembourg)



Five emission sources are identified for the disposal life-cycle stage:
- paper recycling (life cycle stage 8a)
- car shredding sites (life cycle stage 8b)
- municipal incineration stations (life cycle stage 8c)
- municipal land fills (life cycle stage 8d)
- waste remaining in the environment (life cycle stage 8e)

Paper recycling (life cycle stage 8a)

The deinking of recycled paper is assumed to be a potential local release source. According to the emission scenario document (IC-12) in the TGD, between 6 and 90% of the ink can be washed to waste water during recycling. Given the hydrophobic nature of DEHP, the lower value of 6% would appear to be most appropriate. The amount DEHP in recycled paper is corrected for the amount evaporated during end-product use (see section 3.1.1.3, **Table 3.27**) to be 3,322 – 16.7 = 3,305 tpa. Furthermore, during primary treatment, a removal rate of 90% is estimated for insoluble substances. With a paper-recycling rate of 50%, the release to waste water would be $3305 \cdot 0.06 \cdot 0.1 \cdot 0.5 = 9.9$tpa. The continental and regional emissions are set to 90% and 10% respectively (default) and are presented in **Table 3.30**. This amount is used as input for use pattern "6 recov." in the EUSES calculation (see **Table 3.48**) as emission to waste water. The default values for fraction of main source (0.3) and number of emission days (250) are used.

(The occurrence of DEHP in toilet paper has been measured in the Netherlands to be 3-16 mg/kg (Vollebregt et.al. 2000). There was no clear difference between new and recycled papers).

Table 3.30 Distribution and total annual amount of DEHP emissions from paper recycling sites within EU (life cycle stage 8a)

Compartment	Total (tpa)	Continental* 90%	Regional* 10%
Air	0	0,0	0,00
Waste water**	9,9	8.91	0.89
Surface water	0	0	0
Urban/Ind. Soil	0	0	0
Total	**9,9**	**8,91**	**0,89**

* Used as input data for use pattern "6 recov." in the EUSES calculation (see Table 3.48)

** 70% is assumed to be treated in STP and 30% be released directly to surface water (according to TGD)

Car shredder waste (life cycle stage 8b)

Shredding of disposed vehicles is a potential source for release of DEHP (from car-undercoating and cables). Shredding is carried out to separate the non-ferrous from the ferrous metals for recycling purpose. A by-product is the so called light shredder fraction, which contains most of the rigid, flexible and foamed plastics, elastomeres, wood, glass and dirt. This light shredder is land filled within the EU. The process of dry shredding is the most practised system in Europe. Old cars enter the shredding process after removal of fluids, batteries and converter radiators. The shredding itself takes place through hammering on the scraps until the granulation of the scraps is small enough to be pushed through the gates. The light fraction is separated through an aspiration system. During the shredder process a normal temperature was measured ranging

between 30 and 45°C, with exceptionally higher temperature occurring only temporary at compact and large individual items (e.g. crank shafts).

The following calculation for the dry process can be made (INDUSTRY, 1999):

Car processed in Denmark and Germany:	3,850,000
Shredder sites in EU:	252
Car processed in EU:	10,600,000
PVC per car:	16 kg (source APME)
plasticizer per car:	5.4 kg (average of 35% soft PVC)(ECPI)
DEHP per car:	1.1 kg (average of 25% DEHP in soft PVC)
Total EU input of DEHP in shredder:	11,000 tpa
Total input per site:	43.5 tpa = 145 kg/d

As a worst case emission factor for the air the same release factors for "plastisol processing", i.e. 0.05% was assumed:

Local release of DEHP into air	145 kg/d · 0.05% = 0.0725 kg/d (= 0.021 t/300d)
Total releases	5.5 tpa (0.021 · 252)

Uncontrolled releases of particles are also expected to occur to the surroundings. Based on a rough assumption the particle emission during disposal is estimated to be 124 tpa (see **Table 3.35**). It is assumed that 50% (62 tonnes) of these emissions emanates from car shredding sites. Even if particles are released to the air compartment particles are assumed to deposit to the ground. The recipient for this emission is therefore assumed to be the urban or industrial soil.

Release to water is not expected from the dry processing. However, some processing sites separate metals by water flotation. The frequency is however, assumed to be low.

According to Industry use of DEHP in plastisol for cars has declined about 90% since1995. The shredding currently in practice will on average be on cars built pre-1995. Thus this type of DEHP emission will decrease in the future.

The continental and regional emissions are set to 90% and 10% respectively (default) and are presented in **Table 3.31**.

Table 3.31 Distribution and total annual amount of DEHP emissions from car shredding sites within EU (life cycle stage 8b)

Compartment	Total (tpa)	Continental* 90%	Regional* 10%
Air	5.5	5.0	0.50
Waste water	0	0	0
Surface water	0	0	0
Urban/Ind. Soil	61.9	55.7	55.7
Total	**67.4**	**60.7**	**6.2**

* Used as input data for use pattern "8 recov." in the EUSES calculation (see Table 3.48).

The values in **Table 3.30** are used as input data in the EUSES calculation (see Section 3.1.3). In the EUSES calculation the fraction of main source is set to 0.04 (252 sites in EU) and the number of days for emission is set to 300 days.

Incineration of municipal waste (life cycle stage 8c)

Based on statistics from ETSU (1996) the average incineration capacity within EU is calculated to be about 24% (corrected for different populations). The annual releases of DEHP from Danish waste incineration 1992 has been estimated by Miljöstyrelsen by measuring the concentration in smoke, ash, waste water etc. at an incineration station burning municipal waste (Miljöstyrelsen 1996, p.71). The following DEHP concentrations where measured:

Exhaust gas	5.7-17 µg/m^3
Gas cleaning residue product	1,000-5,000 µg/kg
Cleaning water	< 0.5-1.0 µg/l
slag	860-4,000 µg/kg
fly ash	< 400-2,800 µg/kg

Based on these data the following emission can be calculated:

Recipient	Emission	Source
Air	0.118 tpa	Exhaust
Municipal land fill	1.17 tpa	Slag, fly ash, gas cleaning residue product
Water	0 tpa	Exhaust gas cleaning
Total	1.29 tpa	

Based on the incineration capacity in Denmark of 36% (ETSU 1996) the EU emissions can be estimated:

Population of Denmark:	5.3 millions
Population of EU:	370 millions
EU, incineration rate:	24%
Denmark, incineration rate:	36%

Conversion factor = (370 / 5.3) · (24 / 36) = 46.54

With this conversion factor the Danish figures are recalculated to EU emissions (see **Table 3.32**).

The continental and regional emissions are set to 90% and 10% respectively (default) and are presented in **Table 3.32**.

Table 3.32 Distribution and total annual amount of DEHP emissions from incineration stations within EU (life cycle stage 8c)

Compartment	Distribution (%)	Total (tpa)	Continental* 90%	Regional* 10%
Air	9	5.5	4.95	0.55
Waste water	0	0	0	0
Surface water	0	0	0	0
Urban/Ind. soil	0	0	0	0
Munic. Land fill	91	(54.5**)	**	**

* Used as input data for use pattern "9 recov." in the EUSES calculation
** Not included in the EUSES calculation.

Disposal to municipal landfills is not considered as a direct release to the environment and is therefore not used for PEC calculations. This will give a total environmental emission of 5.5 tpa (4.95 + 0.55). The air is the only primary recipient.

The values in **Table 3.33** are used as input data in the EUSES calculation for the use pattern "9 recov." (see Section 3.1.3). In the EUSES calculation the default value on the fraction of main source 0.2 (B-table B5.3) and the number of days for emission is set to be 300 days.

Municipal land fill (life cycle stage 8d)

The municipal landfills are identified to emit DEHP mainly through the leakage water (ECPI 1996b). Data from ECPI are used in this assessment. An estimate of the total emission within EU based on measured data from UK (ECPI, 1996b) gives a leached amount of 15 tpa.

Table 3.33 Distribution of DEHP emitted from municipal landfills (life cycle stage 8d)

Compartment	Total* (tpa)	Continental 90%	Regional 10%
Air	0	0	0
Waste water**	15.0	13.5	1.5
Surface water	0	0	0
Urban/Ind. Soil	0	0	0
Total	**15.0**	**13.5**	**1.5**

* To Table 3.35
** 70% is assumed to be treated in STP and 30% be released directly to surface water (according to TGD).

The low dissipation rate of DEHP in the municipal landfills environment (see Section 3.1.2.2, Landfill conditions) will probably cause accumulation of DEHP in municipal landfills. The future emissions from municipal landfills may therefore be higher.

A landfill is not fitting the local scenario according to TGD. These emissions are therefore only used for the calculations of continental/regional PECs. In the EUSES calculation these emissions are added to the use pattern"1 recov." together with emission from "waste remaining in the environment" (see below).

Waste remaining in the environment (life cycle stage 8e)

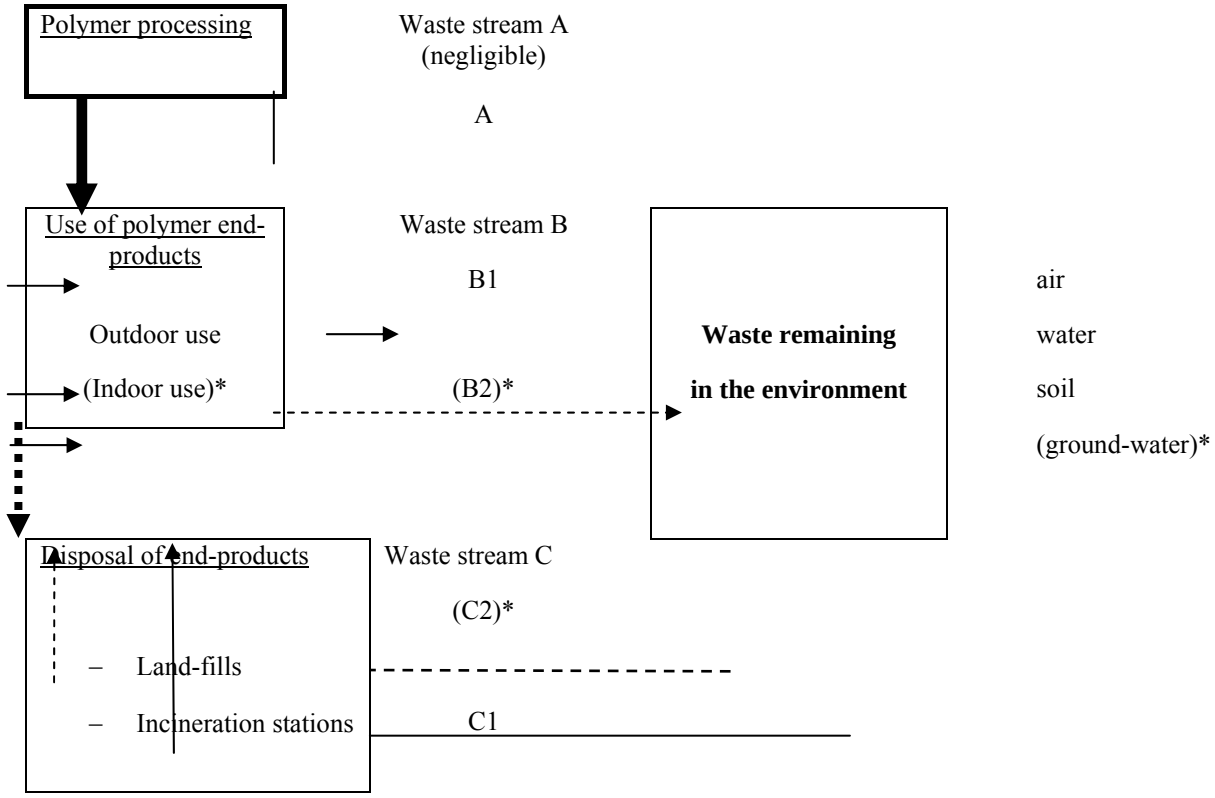
Waste remaining in the environment is expected to be particles/fragments abraded from end-use products during their service life and during disposal (e.g. particles abraded from car undercoating, coil coating, shoe soles and fragments of plastic bags etc.). These particles are primarily released to the urban/ind. soil compartment. However, the smallest fraction may also be distributed to the air compartment or to the surface water environment ending up in the sediment. **Figure 3.3** gives an overview of contributions to this waste type considered in this assessment.

End products used for out-door purpose is the most obvious source for this waste formation. Some data to estimate the abrasion from outdoor use are available. However, for the in-door use except for abrasion of floor (approximately 78% of the consumption of DEHP) data are missing.

Compared to the other life cycle stages in this RAR the emission from waste remaining in the environment is more difficult to estimate. The polymer particles can be assumed to have a certain lifetime. Therefore, it can be assumed that there is a "pool" of polymer particles in the environment that has a far larger volume than the yearly emissions of particle bound DEHP. However, there is no information on the fate of the polymer particles or the releases of DEHP from them. Furthermore, the bioavailability of polymer associated DEHP is not known.

In this assessment the worst case approach is used, i.e. that the release of DEHP associated to polymer particles is treated as an emission of DEHP.

Figure 3.3 Different (polymer) waste stream contributions to "waste remaining in the environment" (waste streams A, B2 and C2 is not included in the calculations)



* only partly included due to lack of method/data

Waste from out-door use

Among out-door use of end-products the following are expected to form waste during use and disposal (waste streams B and C in **Figure 3.3**):

1) Car undercoating
2) Roofing material
3) Coil coating
4) Fabric coating
5) Cables and wires
6) Hoses and profiles
7) Shoe soles
8) Sealant, adhesives etc
9) Paints and lacquers

To estimate the emissions from these types of wastes, the waste formation rate (release of PVC containing DEHP) needs to be estimated from both use (waste streams B in **Figure 3.3**) and disposal (waste streams C).

1. Car undercoating

The waste formation during the service life is assumed to be approximately 10% (waste stream B1, Volvo 1998). For the remaining 90% the release during disposal is assumed to be 2% (no reference). Releases are expected from different waste handling processes (squeezing, shredding) where particles will be released. The emission at the shredding sites is already covered by a special local scenario (life stage 8b, see above) and is assumed to be half of the total particle emission.

2. Roofing material

No data are available on the particulate losses during the service lifetime and during disposal. The waste formation during the service lifetime is assumed to be approximately 5% (no reference). For the remaining 95% the release during disposal is assumed to be 2% (no reference).

3. Coil coating

Losses during the service life has been estimated in a survey on a number of buildings with PVC coil coated roofs in Stockholm 1998 (Rathleff-Nielsen 1999, Skog 1999). This study estimates that about 50% of the coating was lost after 10 years use. The same loss is used in this assessment. For the remaining 50% the release during disposal is assumed to be 10% (no reference).

4. Fabric coating

No data are available on the losses during the service life and during disposal. It is assumed that the waste formation rate is 4% (no reference). For the remaining 96% the release during disposal is assumed to be 2% (no reference).

5. Cable and wire

No data are available on the losses during the service life and during disposal. For over ground applications a loss of 2% is assumed. For the remaining 98% the release during disposal is

assumed to be 2% (no data, worst case assumption). For under ground applications a loss of 2% is assumed. For the remaining 98% the release during disposal is assumed to be 80% (no data, worst case assumption).

6. Hoses and profiles

No data are available on the losses during the service life and during disposal. A maximum of 2% is however assumed to be released during the service life (no ref.). For the remaining 98% the release during disposal is assumed to be 2% (no reference).

7. Shoe soles

No data are available on the losses during the service life and during disposal. 10% is however assumed to be released during the service life (no ref.). For the remaining 90% the release during disposal is assumed to be 1% (no reference).

8. Sealant, adhesives etc.

No data are available on the losses during the service life and during disposal. 5% is however assumed to be released during the service life (no reference). The release during disposal is assumed to be 5% (no reference).

9. Paints and lacquers

No data are available on the losses during the service life and during disposal. The particle emission during service life is assumed to be 5% (no reference). The release during disposal is assumed to be 5% (no reference).

In **Table 3.35** the emissions from different kind of waste remaining in the environment are calculated. When calculating the particle emission from end-products corrections are made for emissions during earlier life cycle stages.

There are no data available about the recipient of emissions from "waste remaining in the environment". However, since the polymers occur as solids they are assumed to end up in the soil and sediment environments. Smaller fractions can be expected to be transported by wind and water to sediments while larger pieces remain in the soil. Compared to the releases from product use the emission to air and water is expected to decrease and the emission to soil increase. In this assessment the emissions to air and water are assumed to be 0.1% and 25% respectively and the emission to soil is assumed to be to 75% (see **Table 3.34**). These figures are used in **Table 3.35** to estimate the environmental distribution of the emissions.

Table 3.34 Environmental distributions of DEHP released from use of end products and waste remaining in the environment

Primary Recipient	Environmental distribution		
	During normal use (%)	From waste remaining in the environment (%)	From soil buried cable (use and waste) (%)
Air	1	0.1	0
Water	49.5	25	0
Soil	49.5	75	100

The calculations in **Table 3.35** show that the annual releases from different waste types vary between 59 to 8,188 tpa. The highest figure is attributed to soil buried cables. This is due to the

assumption that 80% of the cables are left in the ground at disposal. In TGD the urban/industrial soil depth is only 5 cm. The ordinary PEC calculations are therefore not able to cover this release. Development of new distribution models is therefore needed (with focus on groundwater exposure). Until better model are available the contribution from soil buried cables are excluded from the PEC calculations. Excluding soil buried cables the annual releases vary between 59 and 4,337 tpa. Coil coating and shoe soles are the dominating sources.

The uncertainty of these figures is high due to several assumptions and simplifications.

Waste from in-door use

In-door uses of polymer end products will most probably also cause "waste remaining in the environment". The abrasion from floor is already included in the section for end-products use. An obvious life cycle stage that, however, is not included is the demolishing of buildings that contain products like electrical cables, hose profiles, polymer wallpaper, paint, sealants and flooring (Sten 1998). Since such demolished building material may be used for land filling, ground water can be assumed to be exposed. More data and/or new calculation methods are, however, needed before such a life cycle stage can be introduced in the assessment.

Other waste sources

An analysis of domestic organic waste used for producing compost shows that the concentration of DEHP is in the same level as for municipal STP sludge (Kjölholt et. al, 1998). The extent of such use is limited today. However, new EU directives for domestic landfills will probably in the near future increase such uses.

Table 3.35 Out-door use: Calculation of emissions of DEHP from waste remaining in the environment at steady state (life cycle stage 8d)

	unit	Car under coating	Roofing material	Coil coating	Fabric coating	Cable & Wire		Hoses Profiles	Shoe soles	Paint Lacquer	Sealant adhesives	Total
						Air	*Soil*					
Total quantity of DEHP used in applications.	tpa	7,000	1,000	5,000	21,000	4,000	*16,000*	6,000	40,000	724	5,571	**106,295**
Quantity of DEHP emitted from formulation and processing (accounted for elsewhere in assessment)[1]	tpa	26	4	19	79	15	*60*	23	150	27	1,978	**599**
Quantity of DEHP present in outdoor applications.	tpa	6,974	996	4,981	20,921	3,985	*15,940*	5,977	39,850	697	5,374	**105,696**
Volatilisation and leaching loss during service life for outdoor applications (accounted for elsewhere).	tpa	92	49	524	440	126	*5,760*	31	73	199	117	**7,411**
Quantity of DEHP remaining in outdoor applications after volatilisation and leaching losses.	tpa	6,881	947	4,457	20,481	3,859	*10,186*	5,946	39,777	498	5,257	**98,284**
Percentage particulate emission over service life of product.	%	10	5	50	4	2	*2*	2	10	5	5	
Amount of DEHP emitted as waste remaining in the environment over service life of product.	tpa	688	47	2,229	819	77	*204*	119	3,978	25	263	**8,449**
Amounts of DEHP remaining in product at disposal.	tpa	6,193	900	2,229	19,662	3,782	*9,976*	5,827	35,799	473	4,994	**89,836**
% particulate emission at disposal.	%	2	2	5	2	2	*80*	2	1	5	5	

Table 3.35 continued overleaf

Table 3.35 continued Out-door use: Calculation of emissions of DEHP from waste remaining in the environment at steady state (life cycle stage 8d)

	unit	Car under coating	Roofing material	Coil coating	Fabric coating	Cable & Wire		Hoses Profiles	Shoe soles	Paint Lacquer	Sealant adhesives	Total	
						Air	*Soil*						
Amount of DEHP emitted as waste remaining in the environment at disposal[3]	tpa	62	18	111	393	76	*7,981*	117	358	24	250	**9,389**	**To EUSES**
Total amount of DEHP as waste remaining in the environment	tpa	750	65	2,340	1,212	153	*81,858,027*	235	4,336	49	513	**17,838**	**calculation[2]**
Distribution of emission to:													*Soil buried*
Air	%	0.1	0.1	0.1	0.1	0.1	*0*	0.1	0.1	0.1	0.1		
Surface water	%	25	25	25	25	25	*0*	25	25	25	25		*cable*
Urban soil	%	75	75	75	75	75	*100*	75	75	75	75		*excluded*
Total amount of DEHP as wast													
Air	tpa	0.75	0.07	2.34	1.21	0.15	*0*	0.24	4.34	0.05	0.51	**9.1**	**9.1**
remaining in the environment to:													
Surface water	tpa	188	16	585	303	38	*0*	59	1,084	12	128	**2,413**	**2,413**
Soil	tpa	563	49	1,755	909	115	*8,185*	177	3,252	36	384	**15,425**	**7,240**

1) Based on an average emission from polymer processing/formulation of 0.38%
2) To Table 3.36.
3) Car under coating: The total emission from waste disposal of cars is 124 tpa. Half of this emission is assumed to occur at car shredding sites and is therefore removed.

Due to the diffuse nature of the emissions from "Waste remaining in the environment" they are only used for their contribution to the regional scenario. The total diffuse EU emissions (in tpa) from waste remaining in the environment and landfills to different environmental compartments are summarised in **Table 3.36**. The emissions from landfills are also excluded from local PEC calculations. As a simplification the emissions from waste remaining in the environment and landfills are added to the same use pattern ("1. Recov.") in the EUSES calculation. The continental and regional emissions are set to 90% and 10% respectively (default).

Table 3.36 The DEHP emission from landfills and waste remaining in the environment (life-cycle stages 8d+e)

Compartment	Total (tpa)	Continental* 90%	Regional* 10%
Air	9.1	8.2	0.91
Waste water	15	13.5	1.5
Surface water	2,413	2,172	241
Industrial/urban soil	7,240	6,516	724
Total	**9,677**	**8,710**	**968**

* Used as input data for use pattern "1 recov." in EUSES calculationsee Table 3.48.

3.1.1.5 Summary of releases

Sources and recipients

The EU emissions from different parts of the total life cycle are summarised in **Table 3.37** and **Table 3.38**. The releases from industrial point sources are rather low compared to the high volume that is handled. The primary recipients during production, formulation and processing are mainly air and wastewater. Releases during end-product uses are expected to be relatively high. These emissions cause a wide disperse distribution. All types of recipients are exposed for these emissions. The STP is mainly exposed from in-door use and the surface water and soil are mainly exposed from out-door use.

A study by UK water industry research limited suggest that leaching from newly installed plastics in housing is a significant diffuse input to wastewater treatment works, see Section 3.1.4.2. (UKWIR, 2005)

Emissions from car shredding, municipal land fills and incineration stations seem to be in the same low order as from the industrial point sources. The primary recipients are soil (car shredding), the water compartment (municipal landfills), municipal landfills (incineration) and air (incineration). The low dissipation rate of DEHP in the municipal landfill environment will probably cause accumulation. The emissions from landfills may therefore increase in the future.

Considerable emissions in particle form could be expected from end products that are not recycled, incinerated or land filled. Dominating sources are shoe soles, out-door use of coil and fabric coating and in-door floors. The primary recipients are expected to be soil followed by the aquatic compartment (mainly sediment) and to a lesser extent air. The uncertainty of these estimations is, however, high due to limited data.

DEHP in waste formed from buried cables and demolished building material is assumed to cause emission to ground water in urban areas. It was not possible to estimate these emissions (due to limitations in available estimation methods).

The over all distribution of DEHP is 2% to air, 21% to water and 77% to urban/industrial soil. Two thirds of the load to urban/industrial soil comes from cables buried deeper than 5 cm and is therefore not included in the calculation of regional PECs.

Over-all accumulation of DEHP

Large amounts of DEHP in polymers are building up in:

- End products with long service lives (e.g. building material);
- Land fills;
- Waste remaining in the environment (pieces of polymer).

Large amount of polymer end products accumulates in landfills. The content of DEHP will sooner or later emit from the polymer matrix. Emitted DEHP may then be degraded in the landfill or leave it. The amount of DEHP released from a landfill today is assumed to be small. Also the release of the degradation product, MEHP, is expected to be small (Mersiowsky et.al. 1999).

Compared to land fills DEHP in "waste remaining in the environment" is much more out of technical control. Due to high persistency of the DEHP/polymer complex this amount is also expected to still increase in the technosphere. Some polymer end products with service lives of several decades still in use are also accumulating in the technosphere.

As a consequence the amount of DEHP in the technosphere (incl. the waste) is still increasing. Increasing amounts may also cause increasing emissions. With constant consumption and waste management the DEHP levels (and emissions) will after a while reach steady state (when consumed amount = emitted amount + incinerated amount + amount degraded in landfills).

The emissions calculated in this assessment are in some cases dominated by emitting materials with long lifetimes (e.g. soil buried cables). This means that estimated PECs that are dominated by such diffuse sources may to some extent reflect a future hypothetical emission. In other words, the emissions we can expect in the future if we continue to handle DEHP in the same ways as today. This future perspective may cause difficulties in comparing calculated regional PEC values, which reflects a steady state situation with monitoring data. However, DEHP have been in use since approximately 1,940. It has had a relatively stable consumption level in Europe the last 30 years. There are only a few products that have service lives longer than that (e.g. soil buried cables). Therefore, a major increase of concentrations of DEHP in the environment is not to be expected over the coming years if the consumption volume and use pattern stay stable. Thus, the estimated $PEC_{regional}$ should be comparable with monitoring data.

Table 3.37 A summary of the distribution in tonnes (total in EU) of DEHP emissions to different environmental compartments during the total life cycle

Life-cycle stages	Emission Air	Distribution Waste Water[1]	in Urban 0-5 cm	tonnes Soil[3] >5 cm	/year Total
1a production	2	682	7	0	691
1b transportation	0	50	0	0	50
Polymers:					
2a-g Polymer-proc/form	197	197	0	0	394
Non-polymers:					
3a Sealant-formulation	28	111	1	0	140
3b Sealant-processing	1	0	56	0	57
4a Paint-formulation	4	14	0.1	0	18
4b Paint-processing	0	1	7	0	9
5a Ink-formulation	4	17	0.2	0	21
5b Ink-processing	83	0.8	2	0	86
6 Ceramic-formulation	0.1	0.6	0	0	1
Total / industrial uses	**319**	**1,074**	**74**	**0**	**1,467**
7 Polymer-Indoor use	181	1,316	0	0	1,497
7 Polymer-Outdoor use	6	688	642	5,760	7,096
7 Non-pol. Indoor use	18	314	0	0	333
7 Non-pol. Outdoor use	2	157	157	0	316
Total / end-product uses	**207**	**2,475**	**799**	**5,760**	**9,241**
8a Paper recycling	0	9.9	0	0	10
8b Car shredder	5.5	0	62	0	67.5
8c Incineration stations	5.5	0	0	0	5.5
8d Municipal land-fill	0	15	0	0	15
8e Waste / environment[2]	9	2,413	7,240	8,185	17,847
Total / disposal	**20**	**2,438**	**7,302**	**8,185**	**17,945**
Total:	**546**	**5,987**	**8,175**	**13,945**	**28,653**

1) Emission to waste waster except for scenarios 7/out door and 8e (see text for more details)
2) Waste remaining in the environment. Only the contribution from out-door use.
3) Soil buried cables in “urban/Ind. Soil” (>5 cm).

Table 3.38 A summary of the contribution of emissions from different life stages in % of the total DEHP emission

Life-cycle stages	Emission distribution in % of total				
	Air	Waste Water[1]	Urban soil[3]		Total
			0-5 cm	>5 cm	
1a production	0.01	2.4	0.03	0	2.4
1b transportation	0	0.2	0	0	0.2
Polymers:					
2a-e Polymer-proc/form	0.7	0.7	0	0	1.4
Non-polymers:					
3a Sealant-formulation	0.10	0.4	0.004	0	0.5
3b Sealant-processing	0.004	0	0.2	0	0.2
4a Paint-formulation	0.01	0.05	0.0005	0	0.06
4b Paint-processing	0	0.005	0.03	0	0.03
5a Ink-formulation	0.014	0.06	0.0006	0	0.07
5b Ink-processing	0.3	0.003	0.01	0	0.3
6 Ceramic-formulation	0.0003	0.002	0.00001	0	0.002
Total / industrial uses	**1.1**	**3.7**	**0.3**	**0**	**5.1**
7 Polymer-Indoor use	0.6	4.6	0	0	5
7 Polymer-Outdoor use	0.02	2.4	2.3	20	25
7 Non-pol. Indoor use	0.06	1.1	00	0	1.2
7 Non-pol. Outdoor use	0.005	0.6	0.6	0	1.1
Total / end-product uses	**0.7**	**8.6**	**2.8**	**20**	**32**
8a Paper recycling	0	0.03	0	0	0.03
8b Car shredder	0.02	0	0.2	0	0.2
8c Incineration stations	0.02	0	0	0	0.02
8d Municipal land-fill	0	0.05	0	0	0.05
8e Waste / environment[2]	0.03	8.5	26	29	63
Total / disposal	**0.07**	**8.5**	**25**	**29**	**63**
Total:	**1.9**	**21**	**28**	**49**	**100**

1) Emission to waste waster except for the scenarios 7/out door and 8e (see text for more details)
2) Only the contribution from out-door use
3) Soil buried Cables in "urban/Ind. soil" (>5 cm).

3.1.2 Environmental fate

3.1.2.1 Degradation in the environment

3.1.2.1.1 Atmospheric degradation

The knowledge of the fate of DEHP in the atmosphere is limited. Available data indicate, however, that photodegradation is the main degradation pathway in the atmosphere.

Furtmann (1996) found several reports concerning photodegradation in the atmosphere (reaction with OH-radicals, 25°C). Two of the reports concern the vapour phase. Singh et al (1983) had estimated a half-life of 1.2 days and Klöpffer et al (1990) reported a half-life of 0.32 days. A third study, Zetzsch (1991) reported an estimated half-life of 1.07 days concerning the aerosol form. One report (Fraunhofer Gesellshaft 1984 in TSD 1991 or ECETOC 1985) indicated that DEHP is rapidly photodegraded in the atmosphere, with a half-life of less than one day (no further experimental information was available). A half-life of one day is used in this assessment.

3.1.2.1.2 Aquatic degradation

Abiotic degradation

Available studies on abiotic degradation are summarised in Table 4 in Annex 1.

The abiotic hydrolysis of DEHP to mono(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanol is expected to be very slow. An estimated half-life of approximately 2,000 years has been reported (Giam et.al. 1984).

Photooxidation of DEHP in aquatic systems is reported to occur very slowly (TSD 1991). The low photooxidative degradation of DEHP is not surprising since the resistance of DEHP to photo-oxidation in general is one of the characteristics that make it suitable as a plasticizer in durable products (Lundberg and Nilsson 1994).

Leyder and Boulanger (1983) determined UV absorption of 10 different dialkyl phthalate esters including DEHP. They found that the absorption spectrum coincided within the experimental errors. The determined UV absorption coefficients (λ maxim) were 198.5; 229.5; 275.5 and 281 nm. A broad atmospheric window covers these wavelengths up to approximately 1.5 µm (Lillesand and Kiefer 1979).

Wolfe et al. (1980) used a simulation model (Exposure Analysis Modelling System EXAMS) and calculated that the loss via photolysis would be 0% from a river over a period of 1 hour and 1.8% from a pond over a period of 30 days. For a period of 200 days the loss was calculated to 1.4% from a eutrophic lake and 13.7% from an oligotrophic lake.

Available studies on aerobic and anaerobic biodegradation are summarised in Tables 5 - 8 in Annex 1. Studies assessed to be of acceptable quality for the assessment are marked with an asterisk. Studies of unacceptable quality, for example with incomplete study reports or insufficient information on test conditions are indicated by ”r”. Studies referred to in review articles and other secondary literature, i.e. where no original study reports were available are

only briefly summarised (marked with "s"). Since these studies could not be evaluated, the quality and reliability of the data are unknown.

The degradation of DEHP by different micro-organisms and the effects on microbial respiration has been investigated in a number of studies (e.g. Kurane et al, 1978, Engelhardt et al, 1977, Mathur, 1974). The studies do not give any information on the rate of degradation of DEHP under natural conditions, but the results indicate that the substance can be used as a carbon source for a range of microbial species. Several studies also indicate that adaptation is of importance for the degradation rate of DEHP.

Ready biodegradability

Available studies on ready biodegradability are summarised in Table 5, Annex 1.

In one study (Hüls AG 1994 (unpublished)), performed according to OECD Guideline No. 301 B (Modified Sturm-test), DEHP is shown to be "readily biodegradable" (82% after 28 days) and also fulfil the "10-days window" criteria (see **Table 3.39**). Test concentration of DEHP was 20.3 mg/l (dissolved in Vestinol) and temperature was 20-22°C.

Table 3.39 Modified Sturm-test (Guideline No. 301 B)

Exposure days	% degraded	
	Replicate 1	Replicate 2
4 days	0	1
8 days	0	35
14 days	30	67
18 days	63	76
22 days	72	73
25 days	80	84
28 days	80	81
29 days	78	85

The different biodegradation results in the two replicates can probably in part be explained by the high hydrophobicity that makes it difficult to dose the chemical in a bioavailable form (Scholz 1998, oral communication).

The biodegradation of DEHP was investigated in sludge from the STP of BASF AG (BASF AG 1984). The test procedure was a Modified Respiration Test (BOD of ThOD) according to EG-Guideline 79/831. Test concentration was 100 mg DEHP/l and 10 mg/l emulsifying agent (Nonyl-phenol, 10 EO + 5 PO). The test was performed at 20°C. After 28 days of incubation, 60-70% of the original DEHP was degraded.

In a manometric respirometry test (OECD 301F), the biodegradation was studied in fresh sludge from a domestic STP. The test concentration of DEHP was 45.6 mg/l. The temperature was not reported. Biodegradation started after a lag-phase of 8 days, and after 28 days 63% was degraded.

In a CO_2 evolution test, performed by Struijs and Stoltenkamp, 1990, the degradation of DEHP was studied in sewage from a local STP. Test concentration was approximately 35 mg C/l. After 28 days at 20°C, only 4 – 5% was mineralised.

A study performed with river water and 1% domestic sewage (Waggy, 1972) and a test concentration of 33.3 mg/l, resulted in 54% mineralisation after 33 days. Test temperature was not reported. In another study (Freitag et al, 1985), only 4% of the applied amount was degraded after 5 days. However, several deficiencies in the study report, such as insufficient information on temperature or other test conditions, makes the results of less value.

Inherent biodegradability

Available studies on inherent biodegradability are summarised in Table 5, Annex 1.

In two studies with activated sludge obtained from municipal sewage treatment plants (Saeger and Tucker, 1976; O'Grady et al, 1985) the degradation rate of DEHP was high. The studies were carried out using a modified 24 hours SCAS procedure. After 24 hours, 70 – 85% of the original DEHP was transformed.

Sugatt et al, 1984 investigated the primary degradation and mineralisation of DEHP in a shake flask biodegradation test. The chemical was exposed to an inoculum, prepared from soil and sewage micro-organisms (1 g soil, 2 ml sewage, 50 ml raw influent sewage, 1 litre mineral salts medium), and adapted to phthalic acid esters for 2 weeks, followed by filtration through glass wool prior to the test initiation. The nominal concentration of DEHP was 20 mg/l in the test solution (100 ml inoculum + 900 ml distilled water). Triplicate test flasks were incubated on a shaker in the dark at 22°C for 28 days. Evolved CO_2 was trapped in an attached reservoir containing barium hydroxide, which was periodically sampled and titrated to measure evolved CO_2 to be compared with the blank. At the start, middle and end of the test, the entire contents of four replicate flasks were extracted with hexane and analysed by GC-FID to determine the primary degradation.

After 28 days, > 99% of the nominally applied DEHP was primarily degraded, while 73 – 92% (mean 86%) was mineralised, with a lag-phase of approximately 2 days.

The influence of adaptation of micro-organisms was demonstrated in a study by Tabak et al, 1981. The original investigation of degradation in unadapted sludge (settled domestic wastewater), resulting in no degradation, was followed by studies on first, second and third subculture degradation. The degradation rate was enhanced for each subculture; in the third subculture approximately 95% was eliminated after 28 days.

Anaerobic degradation

The anaerobic degradation of DEHP in activated ("digested") sludge (undiluted and diluted to 10% in anaerobic mineral salt medium) was investigated by Shelton et al, 1984. Nominal test concentration in the undiluted sludge was 20 mg/l (in diluted sludge, not reported). Test bottles were prepared in duplicate for undiluted sludge and in triplicate for diluted sludge, and were incubated at 35°C. Samples were withdrawn periodically for 10 weeks. Dried samples from undiluted and diluted sludge were extracted with dichloromethane and hexane, respectively, prior to analysis with GC-FID.

No significant degradation of DEHP was observed in the undiluted or diluted sludge during the 10 weeks of incubation.

Ziogou et al, 1989 investigated the degradation of DEHP in anaerobic municipal sewage sludge. Test concentration was approximately 4 mg/l in the first stage of the study, where the samples were kept at 37°C without shaking for 32 days. During a second stage of the study, different concentrations were tested; 10, 1, and 0.5 mg/l (about 30 – 600 mg/kg dwt), and the samples

were shaken during the incubation. GC equipped with electron capture detector took samples regularly for extraction (with dichloromethane) and analysis.

In both stages of the study, DEHP was shown to be persistent to degradation - no decrease in DEHP concentration was observed during the 32 days under anaerobic conditions.

Horowitz et al, 1982 investigated the anaerobic degradation of DEHP in two different sewage sludges at 35C°. A 10% sludge inoculum was amended with 50 ppm carbon of the test compound (duplicate samples). Degradation was monitored by comparing the methane production in DEHP amended sludge and the untreated control sludge during 8 weeks. No difference in methane production could be observed between treated and control samples.

The biodegradation of DEHP under anaerobic conditions was also investigated by O'Connor et al, 1989. Activated (digested) sludge, obtained from a municipal wastewater treatment plant, was diluted to 10% (v/v) in a mineral salts medium. The test concentrations of DEHP were 20, 100 and 200 mg/l. The test bottles were incubated in the dark at 37°C for 140 days. The degradation of DEHP was measured by monitoring of the methane production regularly. Mass balance on the conversion of organic carbon to carbon dioxide and methane were based on the stochiometry of conversion for DEHP ($C_{24}H_{38}O_4$ +12.5 $H_2O \rightarrow$ 8.25 CO_2 +15.75 CH_4), corrected for background levels in control samples. The residual DEHP was determined by UV scan at the end of incubation after 140 days. The number of replicates was not given.

The methane production pattern in DEHP amended samples was identical to that of the background control. The residual DEHP concentrations after 140 days were 0, 31 and 46% of the nominal initial levels (20, 100 and 200 mg/l, respectively). However, the method of analysis of DEHP, and the value of the results are questionable.

Ready-, Inherent-, Anaerobic degradation - Summary and discussion

The results from available studies on ready biodegradability vary from 4-5% mineralisation to 60–86% mineralisation after 28 days. The partly great differences obtained in the different tests on ready biodegradability may be caused by difficulties relating to the low solubility of the test compound. The origin of the inoculum used may also be an important factor, since adaptation to the test compound is indicated to be of great importance for the test results (Tabak et al, 1981). Due to the widespread use of DEHP, a major part of the domestic STP sludge can be expected to be adapted to the substance, and the strict requirement of unadapted inoculate for ready biodegradability testing may be difficult to fulfill.

In screening tests on inherent biodegradation, results ranged from > 95% mineralisation in 5 days in industrial sludge (HOECHST AG 1980) to 86% degradation in 28 days (Sugatt et al, 1984). Results from modified SCAS tests and from studies with inoculate obtained from SCAS procedures, indicate a rapid mineralisation of DEHP (Saeger and Tucker, 1976; O'Grady, 1985). However, in TGD, no specific criteria are developed for positive results in SCAS tests, since the conditions in these tests are optimised for biodegradation.

Under anaerobic conditions, the screening data indicate that DEHP is persistent to biodegradation in sludge.

For the estimation of biodegradation rate in STP to be used in this assessment, DEHP is assumed to be readily biodegradable, since several test results has demonstrated rapid degradation, and since in reality, most industrial and domestic STP that receive DEHP are probably adapted to the substance. The rate constant for biodegradation in STP is set to 1(h^{-1}), in accordance with TGD.

Surface water

Available studies on degradation of DEHP in surface waters are summarised in Table 6, Annex 1.

Saeger and Tucker (1976) studied the transformation (primary degradation) of DEHP in river water from Mississippi. Test concentration was 1 mg DEHP/l in 200 ml portions of river water. The bottles were kept at room temperature. Samples were taken after 7, 14, 28 and 35 days for analysis by means of GC-FID. After 35 days, 35% of the original DEHP was unaltered.

Ritsema et al (1989) studied the biotransformation (primary degradation) of DEHP at 4°C and 20°C in river water from Rhine. Test concentration was 3.3 µg/l. Samples were taken on 0, 1, 3, 7 and 10 days after the start and were analysed by means of GC-ECD. Recovery of the method of analysis was 83 – 97%, limit of detection 0.1 µg/l. During the 10 days of incubation, no degradation occurred at 4°C, while approximately 33% of the parent compound was transformed at 20°C.

Subba-Rao et al (1982) investigated the mineralisation of ^{14}C-DEHP in filtrated lake water from one eutrophic and one oligotrophic lake in USA. Test concentrations were 0.02, 0.2, 2.0 and 200 µg/l. The incubation flasks were kept in the dark at 29°C without shaking. Samples were taken at intervals for 40 and 60 days, respectively. Generated $^{14}CO_2$ was removed by bubbling air through the solutions, after acidification with concentrated sulphuric acid. The remaining radioactivity in the solutions was measured in triplicate by LSC.

After 40 days of incubation, 35 – 71% of the original DEHP was mineralised in the eutrophic lake water. The mineralisation half-lives in eutrophic water could be calculated to approximately 22-64 days at 29°C based on first order kinetics. No detectable lag-phase was observed, and the mineralisation did not seem to be concentration-dependent. In the oligotrophic lake water, no mineralisation occurred during the 60 days of incubation.

In a study reported by Furtmann, (1993) the degradation of DEHP and other phthalates in Rhine water was investigated. The background concentration in the water was approximately 0.4 µg/L, and the samples were amended to concentrations of 2 and 5 µg/L, respectively. Initially, degradation was rapid (at 20°C), with 90% primarily degraded after 8 days. However, at a concentration close to the background, a threshold level appears to have been reached, below which no further degradation was observed. In parallel samples kept at 4°C, no degradation occurred during the incubation period.

Surface Water - Summary and discussion

Most of the available studies on degradation of DEHP in surface waters concern primary degradation. Since the main metabolite MEHP (as identified in several studies) is ecotoxicologically relevant and considered even more toxic than the parent compound, the estimation of the biodegradation rate could preferably not be based on primary degradation. Mineralisation was investigated in one study (Subba-Rao et al., 1982). In oligotrophic water, no mineralisation was observed during 60 days; while in eutrophic waters 35 – 71% was mineralised at 29°C after 40 days.

In two of the studies on primary degradation (Ritsema et al., 1989; Furtmann, 1993), different temperatures were tested. The results indicate that the degradation rate is highly temperature-dependent, since no degradation of DEHP took place at 4°C.

Sediment

Available studies on degradation in sediment are summarised in Table 7, Annex 1.

Johnson and Lulves (1975) studied the aerobic and anaerobic biodegradation of ^{14}C -DEHP at 22°C in sediment from a freshwater pond. Ten grams (WWT) of sediment were covered with 20 ml of pond water. The concentration of DEHP in the systems was 1 mg/l (corresponding to 2 mg/kg wwt). Triplicate samples (plus control) were taken for radiometric analysis after 1, 5, 7 and 30 days. Degradation products were identified by means of TLC and LSC. After 14 and 30 days of aerobic incubation, 53% and 41% of the original radioactivity was recovered in the samples, respectively. The only residue identified, besides DEHP, at the end of incubation was MEHP, accounting for 2% of recovered radioactivity. Approximately 60% of total applied radioactivity was mineralised after 28 days of incubation. During anaerobic conditions, no transformation of DEHP was observed.

Johnson, Heitkamp and Jones (1984) investigated the influence of factors such as test concentration, temperature, pre-adaptation and anaerobiosis on the biodegradation of carbonyl- and ringlabelled ^{14}C-DEHP in freshwater sediments. Four concentrations of carbonyl-^{14}C DEHP were used at 22°C: 0.0182, 0.182, 1.82 and 10 mg/l, corresponding to 0.0182, 0.182, 1.82 and 10 mg/kg wwt in the sediment. Four different temperatures were tested; 5°C, 12°C, 22°C and 28°C. The effects of pre-adaptation and anaerobiosis were investigated at 22°C. In the investigation on anaerobiosis, ringlabelled ^{14}C -DEHP was used. All samples were taken in triplicate. The results are summarised in the **Table 3.40**.

Table 3.40 Degradation of DEHP in freshwater sediment (Johnson, Heitkamp and Jones 1984)

Investigation	Temperature	Test Conc. (mg/l)	Percent (%) Biodegraded Incubation time (days)			
			7	14	21	28
Test conc. (mg/l)	22°C	0.0182	2.2	5.2	7.8	10
		0.182	1.8	4.6	7.0	9.3
		1.82	1.7	4.3	6.5	8.5
		10	3.8	9.1	15	20
Temperature	5°C	Not given	0.0	0.0	0.1	0.8
	12°C		0.6	1.0	2.0	2.5
	22°C		0.8	2.0	4.0	5.5
	28°C		3.1	6.5	9.0	11
Pre-adaptation	22°C	0.0182+acetone	2.5	5.6	8.2	10
(for 28 days)		0.0182+acetone, no adaptation	2.2	4.9	7.1	9.0
		0.0182, no acetone, no adaptation	12	17	21	24
Aerobic/	22°C	0.0143	5.1	9.1	12	14
Anaerobic			1.0	3.2	5.7	9.9

The results indicate that the degradation of DEHP is significantly influenced by test temperature. Concerning pre-adaptation, pre-adapted samples were different from the control, but not significantly different from the solvent control, and the results are difficult to interpret. In this study, degradation under anaerobiosis does not seem to be significantly different from aerobic

degradation. However, no information was given on the methods for achieving oxygen free conditions.

In a study on the effects of five different concentrations of DEHP (25 -– 400 mg/kg wwt) on the respiration rate in a water/sediment system (Larsson et al, 1986), the residual concentration of DEHP was measured after 28 days incubation at 5°C. The recovered DEHP after 28 days ranged between 76 and 134% of the nominal initial concentrations, i.e. little degradation had occurred.

The anaerobic degradation of DEHP in sediment was investigated by Horowitz et al, 1982. Pre-incubated (24 hours) duplicate or triplicate sediment samples from a hypereutrophic lake were treated with DEHP at concentrations of 40 or 240 mg/l. The samples were incubated at 20°C in the dark with occasional shaking for 8 weeks. The degradation of DEHP was monitored by measurement of gas production and by analysis for residual DEHP by GC-FID, after extraction with methylene chloride. After 8 weeks of incubation, no degradation of DEHP had occurred, and formation of methane and carbon dioxide was not different from that in the control.

Sediment - Summary and discussion

Since > 50% degradation was observed only in one study (Johnson and Lulves, 1975), the environmental half-life in sediment is difficult to estimate from available data. However, it is obvious that temperature and oxygen conditions are of major importance for the degradation rate. In studies performed at 5°C (Johnson et al, 1984; Larsson et al, 1986), no degradation took place, and at 12°C only 2.5% was degraded after 28 days. Under anaerobiosis, no degradation was observed.

In conclusion, the degradation rate of DEHP in sediment must be considered to be very slow under environmentally relevant conditions. In the temperature range between 5 and 12°C, and in the anaerobic layers of the sediment, biodegradation is not significant. Assuming a half-life in aerobic sediment (which accounts for 10% of the upper 3 cm layer, TGD default) of 300 days as a rough estimate, would give an overall half-life of 3,000 days in the sediment compartment.

3.1.2.1.3 Degradation in soil

Available studies on degradation in soil are summarised in Table 8, Annex 1.

Schmitzer et al (1988) investigated the degradation of DEHP in suspended soil. A mixture of 250 g of soil and 250 ml of water was shaken for 5 days at 22°C. After this equilibration, 14.6 µg of carboxyl-labelled ^{14}C-DEHP was added (corresponding to 58 mg/kg dwt soil), and the mixture was shaken for additionally 33 days. Organic volatiles and $^{14}CO_2$ was trapped and analysed by means of LSC. During the 33 days of incubation, 22% of applied radioactivity evolved as $^{14}CO_2$. In the same study, dissipation of DEHP in soil/plant systems was investigated. In a closed laboratory system, 8.2% of applied radiocarbon was mineralised after 7 days. In an outdoor lysimeter study, 6.9% of the applied ^{14}C was recovered in soil 111days after application, while 0.51% was found in the leachate at 60 cm depth. The temperature was not reported for the soil/plant systems.

In another study, Dörfler et al (1996) studied the mineralisation of DEHP in three different soils, freshly collected from Greenland (pH 7.2, org. C 2.6%), spruce forest (pH 3.4, org. C 5.4%) and mixed forest (pH 4.5, org. C 5.4%), respectively. The soils were treated with DEHP to a concentration of 0.5 mg/kg or 10 mg/kg WWT and incubated at room temperature for between 28 and 63 days. The moisture content and pH were kept at their natural levels at the time of

sampling. Incubations were also run at lower temperatures (below 10°C). Volatile ^{14}C was trapped and analysed by LSC. At the higher concentration of DEHP, 32, 30 and 22% of the initial concentrations were mineralised at room temperature in the three soils, respectively. At the lower concentration the corresponding figures were 43 - 58% in 63 days. The mineralisation of DEHP was not significant below 10°C and negligible below 3°C.

Rudel et al, 1993 studied the degradation of radiolabelled DEHP in the laboratory at 20°C (according to BBA Guideline 1996) and under simulated outdoor conditions in two different soils. An outdoor lysimeter study with the same soils was also performed. The standard laboratory study was performed at 20°C and 40% of maximum water holding capacity. In the simulated outdoor study, temperature and soil moisture were varied during the test period, and the mean test temperature was 13.5°C. Initial concentration of DEHP was 1 mg/kg WWT. At intervals during the 100 days of incubation, amounts of evolved $^{14}CO_2$, extractable residues, non-extractable residues, and the concentrations of DEHP and degradation products (MEHP and phthalic acid) were determined (on days 0, 8 and 32 in duplicate). The outdoor lysimeter studies were performed with undisturbed soil columns (20 cm i.d., 40 cm in height) that were kept under natural outdoor conditions (mean temperature 13 and 14°C, respectively). The lysimeter soil was divided into three layers (0-5 cm, 5-10 cm, and 10-20 cm) for analysis of parent compound. The soil samples were extracted with acetone/hexane and analysed with LSC. Identification of residues was performed by reversed phase HPLC and GC/MS.

Bound residues accounted for 9.7 and 40% of the initial radioactivity after 100 days in the laboratory study at 20°C, while 2.6 and 47% was mineralised. No other metabolites of DEHP were detected. In the simulated outdoor study, the half-lives of the parent compound were calculated to 31 and 170 days, while in the outdoor lysimeters, DT_{50} were determined to be 14-200 days based on dissipation of the parent compound. Insignificant residues were recovered below the 0 - 5 cm layer in the soil profile.

The fate of DEHP in three calcareous soils from New Mexico was investigated by Fairbanks et al, 1985. The soils were unamended or amended with sewage sludge just prior to or 8 weeks in advance of the tests at a rate of 20 g/kg soil. Test concentration of DEHP were 2 and 20 mg/kg (dwt) soil, added drop wise to 50 g portions of dry soil and incubated for 146 days at 21-25°C. Mineralisation was measured by monitoring of evolved $^{14}CO_2$, trapped in NaOH. The soils were analysed for ^{14}C at termination of the incubation period. The resulting half-lives for DEHP was 8-72 days in the different soils and conditions. In general, DEHP persistence was reduced by previous exposure of the soil to sludge, but was increased or unaffected by fresh additions of sludge. DEHP was more persistent at 20 mg/kg than at 2 mg/kg.

In a study by Kirchmann et al, 1991, the effects of DEHP on soil respiration and nitrogen conversion, and the primary degradation of DEHP was investigated. Soil samples of 10 g each, treated with 5 or 250 mg DEHP/kg dwt, were placed in glass tubes, wetted to ”optimal” moisture content and incubated for 80 days at 25°C. The DEHP content were analysed by GC after extraction with n-hexane and water/ethanol at intervals during incubation. At the test concentration of 250 mg/kg, 50% of the parent compound remained after 80 days, while 20% was unaltered after 80 days at 5 mg/kg. The corresponding half-lives could be calculated to 80 and 30 days, respectively.

In a study performed by Efroymson et al, 1993, the effects of different organic solvents or inorganic nutrients on the degradation of ^{14}C-DEHP in soil were investigated. Duplicate portions of moist soil (silt loam, pH 4.9, 3.8% org. mtr.) equivalent to 50 g of dry weight were amended with 1.0 μg DEHP/g dwt in either 0.5 ml of a test organic solvent (heptamethylnonane,

cyclohexane, hexadecane or dibutylphthalate) or in 50 - 100 µl of dichloromethane, which were then allowed to volatilise. In parallel samples also N and P were added to the soil (in 5.0 ml water containing 4.5 mg K_2HPO_4 and 1.0 mg NH_4NO_3), to determine whether the mineralisation of DEHP could be enhanced by the presence of these inorganic nutrients. Evolved $^{14}CO_2$ was trapped in NaOH and analysed with LSC.

In the presence of organic solvents 1 - 25% of the initial DEHP applied to the soil was mineralised after 20 days, compared to 50% with DEHP alone. The mineralisation was not enhanced by addition of N and P to the soil. Since no test temperature was reported, no valuable conclusion on degradation rate can be made from this study. However, the results points out that the presence of organic solvents in the soil can depress the degradation of DEHP.

The aerobic and anaerobic transformation of DEHP in soil was investigated by Shanker et al, 1985. Triplicate soil samples of 10 g dry weight were amended with DEHP in 0.1 ml of methanol to give a final nominal concentration of 500 mg/kg dwt soil. Anaerobiosis was achieved by flooding with sterile water at least to the double height of the soil columns. The samples were kept at 30°C for 30 days. Samples with autoclaved soil were run in parallel as controls. Residual DEHP and produced phthalic acid were monitored regularly by HPLC after extraction of samples with methanol. Evolved CO_2 was not measured.

After 30 days of aerobiosis, 8.3% of the parent compound measured on day 0 was recovered in the soil, while 1% of the initial DEHP was recovered as phthalic acid. Under anaerobic conditions, 66% of initial DEHP was unaltered after 30 days, and 11% was found as phthalic acid. However, the flooding of soil with sterile water can be suspected not to be a sufficient measure to achieve total anaerobiosis.

Madsen et al., 1999, studied the mineralisation of DEHP in a sandy loam soil with and without amendment of sewage sludge. The soil used had not previously received sewage sludge. DEHP and [ring-UL- ^{14}C] DEHP was added to the soils to concentrations of 1.6, 3.2, 9.9 or 35.1mg DEHP/kg dwt. Soil and sludge amended soil (soil sludge ratio 58:1 (dwt:dwt)) was incubated in the dark at 5,10 or 20^{o}C under aerobic conditions. The water content of the soils was held at 75% of the field capacity. The DEHP mineralisation in sludge-amended soil under anaerobic conditions at 20^{o}C was also studied. The $^{14}CO_2$ produced during incubation was trapped in 1N KOH and quantified by Liquid Scintillation Counting.

Mineralisation of DEHP could be divided into two distinct phases: an initial phase that followed first order kinetics and a slower second phase that was better described by fractional power kinetics. Mineralisation did not reach 50% during the initial phase in any of the measurements. The authors of the study therefore suggest that the effective half time should be based on the kinetics of the second phase. The half times for mineralisation of DEHP in soil was 224 days at 5^{o}C, 187 days at 10^{o}C and 73 days at 20^{o}C. The half times for mineralisation of DEHP in sludge amended soil was >> 365 days at 5^{o}C, 337 days at 10^{o}C and 150 days at 20^{o}C. Mineralisation of DEHP increased with increasing DEHP concentrations in sludge-amended soil. The half time and the duration of the two phases were however not affected by the DEHP concentration. Anaerobic mineralisation of DEHP at 20^{o}C in sludge-amended soil was approximately 5 times slower than aerobic mineralisation and was comparable to the aerobic mineralisation at 5^{o}C.

The mineralisation of a number of organic contaminants, including DEHP, in sludge amended soil was studied by Gejlsbjerg et al., 2001. A coarse sandy soil was used to study the effects of different sludge: soil ratios and different water contents on the mineralisation. In addition the effects of soil type on the mineralisation was studied using the coarse sandy soil and two other soils: a sandy soil and a more clayey soil. The comparison was done with both sludge amended

soil (sludge: soil ratio 1:100 dw) and unamended soil. Uniformly ring–labelled ^{14}C-DEHP and unlabeled DEHP was added to dewatered activated sewage sludge. The sludge was then mixed with soil to achieve sludge: soil ratios of 1:20 (dwt:dwt) or 1:100 (dwt:dwt). Water was added to the mixtures to achieve either 40 or 80% of the water-holding capacity. The soil samples were incubated in the dark at 15oC for two months. $^{14}CO_2$ produced during the incubation was trapped in 0.5 N KOH.

The mineralisation of DEHP in the coarse soil during two months amounted to approximately 20% of added ^{14}C in all treatments regardless of sludge: soil ratio and water content. The mineralisation in the two other soils was lower and was 5.8 and 6.8% of added ^{14}C in sludge amended soil and 8.5 and 9.4 added ^{14}C in unamended soil.

Soil - Summary and discussion

The results from studies on the degradation rate of DEHP in soil are very variable and are difficult to interpret. The range is from 92% degraded after 30 days at 30°C to 3% mineralisation after 100 days at 20°C. As previously mentioned, it is not appropriate to calculate the environmental half-life based on primary degradation rates, since the main degradation product MEHP is of toxicological relevance. Besides, unidentified "bound residues" account for a significant part of the recovered residues.

The overall available data indicate a slow mineralisation rate in soil under environmental conditions. A rough estimate of the soil half-life could be 150 days at room temperature, or approximately 300 days at 10°C, using Q_{10}=2. This estimate is supported by the two recent Danish studies (Madsen et al., 1999 and Gejlsbjerg et al., 2001) were mineralisation in soil and sludge amended soil was studied.

Landfill conditions

Recently, a thesis of Ejlertsson (1997) was published in which the anaerobic degradation of phthalic acid esters was studied under simulated municipal landfill conditions. Both methanogenic and acidogenic conditions were studied in 100 litre lysimeters containing 35-40 kg waste material from a municipal landfill. The conditions were optimised by milling the waste, the temperature was increased and the water content was increased.

Study 1: DEHP and the transformation product MEHP were added to samples from a methanogenic landfill model working in the phase of stable methanogenesis (37°C). MEHP was readily degraded via 2-ethylhexanoic acid to methane and carbon dioxide. DEHP remained unaffected throughout the experimental period (330d).

Study 2: PVC carpet material containing Butylbenzylphthalate (BBP) and DEHP was added. During 32 months incubation period samples (PVC and leachate water) for analysis were collected. Both DEHP (6-216 µg/l) and MEHP (52-3,530 µg/l) occurred in the leachate water. The occurrence of MEHP shows that DEHP was degraded. A hypothesis was proposed that organisms that degrade the more easily degradable BBP by cometabolism also enhance the degradation of DEHP. Another possible explanation proposed is that some organic matter in the waste functions as a solubiliser for DEHP, thus stimulating degradation. After the exposure period the DEHP content in different types of carpet materials were analysed. The result indicates that the emission from PVC in a municipal landfill is a very slow process and decrease with decreasing surface area exposed to micro-organisms. The losses of DEHP were lower during acidogenic condition compared to methanogenic condition.

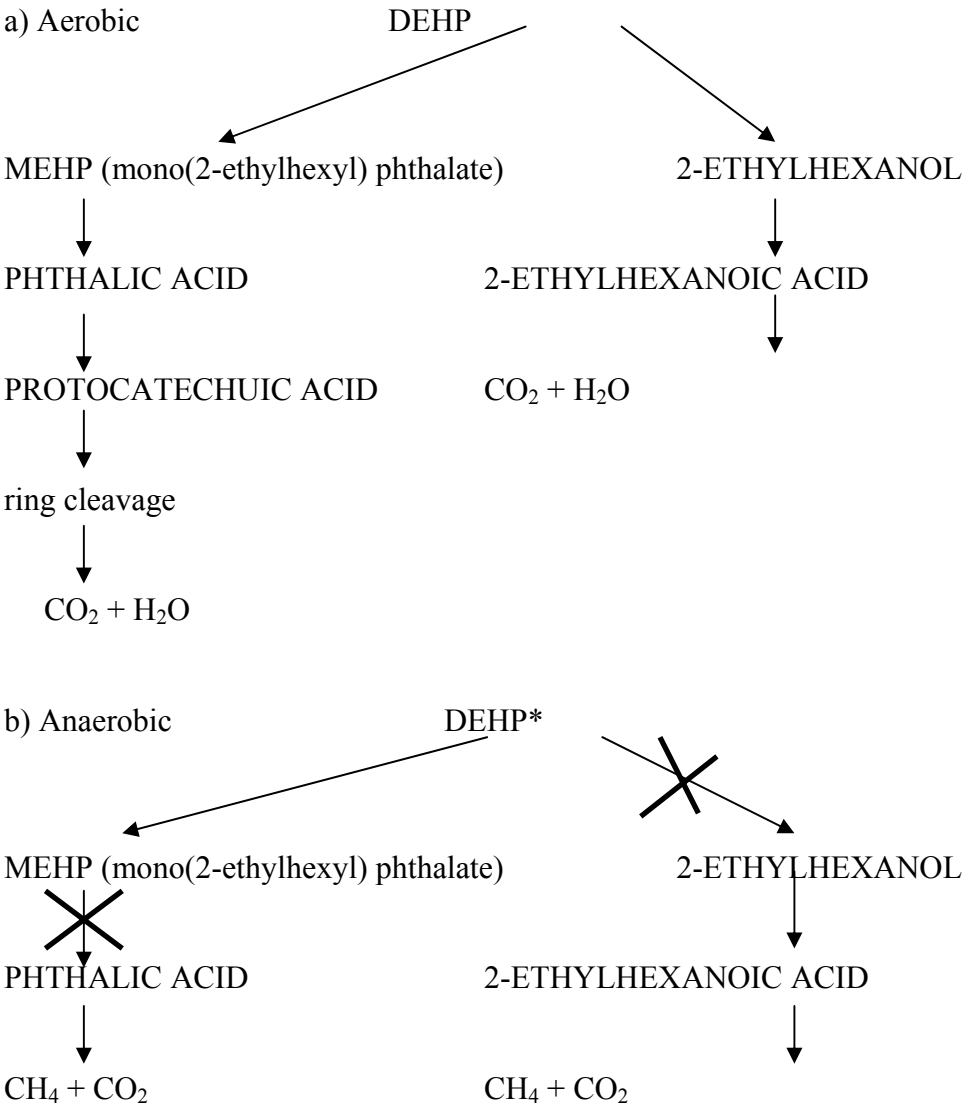
It can be concluded that DEHP can be degraded in the anaerobic condition occurring in a landfill. The mechanisms for degradation and emission from PVC are, however, still unclear. The main metabolite MEHP can be degraded further in anaerobic conditions but also leave the landfill by the leakage water. However, in a monitoring study made by Mersiowsky et.al. 1999 the MEHP concentrations in leakage water were lower than the concentration of DEHP (see Table 14 in Annex 1).

3.1.2.1.4 Degradation pathway

The first part of the aerobic microbial degradation (ester cleavage) of DEHP can be described as a two step reaction: esterases with low substrate specificity (Taylor et al. 1981) causes initial hydrolysis of the ester linkage followed by a rapid decarboxylation of the exposed carbonyl groups of the phthalic acid moiety (Johnson et al. 1984). This reaction is catalysed by either acids or bases (Kice and Marvell 1974). The first major product is the monoester mono (2-ethylhexyl)phthalate (MEHP) (Johnson et al. 1984) and is converted into phthalic acid (Engelhardt et al. 1975 in WHO 1992) and 2-ethylhexanol. According to Kurane et al. (1980) further degradation under aerobic conditions is following the pathway for benzoic acid, i.e. degradation to CO_2 and H_2O via pyruvate and succinate. The 2-ethylhexanol is further oxidized to 2-hexanoic acid. The limiting step in biodegradation (i.e. the step which determines the rate) seems to be the hydrolysis of the second alkyl chain (Løkke 1988). Occurrence of MEHP in leaching water from landfills (Mersiovsky et. al. 1999) shows that the further degradation of MEHP is not complete in a landfill. This incomplete degradation of DEHP is also shown in a study by Toshinori et al. (1996) where MEHP was identified in water extracts from a compost mix made by activated sludge, compost and coffee residue (4:5:1).

In anaerobic conditions in the environment the mineralisation of DEHP is expected to be very slow. The anaerobic persistence seems to be limited to the first degradation step to form the monoester (see **Figure 3.4**). Recent studies by Ejlertsson (1997) show that DEHP can be degraded in anaerobic landfill conditions if also a more easily degradable phthalate is present. The mechanism for this is not clarified. At least in laboratory the primary degradation products 2-ethylhexanol and MEHP are further degraded in anaerobic conditions (see **Figure 3.4**) (Ejlertsson 1996) to methane and carbon dioxide, initially following the same pathway as under aerobic conditions (Ejlertsson 1996, Ejlertsson et. al. 1997).

Figure 3.4 General degradation pathway of DEHP



* Under anaerobic conditions, the first degradation step is inhibited

3.1.2.1.5 Summary of environmental degradation

Based on available studies a half-life in the atmosphere of 1 day is decided to be used in the EUSES calculation. Since the hydrolysis and photolysis in water is very slow the worst case default half-life selected by EUSES (10^6 d for both) is not changed.

The conclusion on biodegradability is used in the EUSES model to calculate realistic worst-case mineralisation rates (DT_{50}) in STP. The conclusion readily biodegradable gives according to TGD a DT_{50} value for STP of 0.029 days. This value is used in the assessment. For a readily biodegradable substance, the TGD default DT50 for surface water is 15 days. However, for surface water experimental data are available indicating slower degradation. In eutrophic lake water 35–71% was mineralised at 29°C after 40 days. Based on this a half-life of 50 days, which is the value suggested by TGD for readily degradable substances failing the 10 day window, is chosen for the calculation of PEC for surface water.

According to TGD, chemicals bound to solid phase are not degraded. Due to a high adsorption the EUSES model calculates a DT_{50} of 30,000 days in aerobic sediment. However, from available data on degradation in sediment, an overall half-life of 3,000 days in sediment was estimated (300 days in the upper aerobic 10% of the sediment, no degradation in anaerobic sediment). These data are used in the calculation of PEC for sediment.

The default values as given in TGD and the estimated half-lives based on available data are given in the **Table 3.41** below.

Table 3.41 Selected biological degradation half-lives for DEHP used in EUSES calculation

COMPARTMENT	DT_{50} default*	(days) used	BASED ON:
STP	0.029	0.029	default
Surface water	15	50	experimental data indicates that the default DT_{50} for readily biodegradable substances failing 10day window is more appropriate.
Agricultural soil	3,000	300	experimental data + temperature correction (Q10=2)
Aerobic sediment	30,000	300	experimental data
Anaerobic sediment	infinite	Infinite	default + experimental data
Sediment, overall	300,000	3,000	default (10 times of the half-life for aerobic sediment)

* Based on Classification: "Readily biodeg." (according to TGD)

3.1.2.2 Distribution

3.1.2.2.1 Adsorption

Available studies on adsorption/desorption are summarised in Table 1, Annex 1.

With a log Kow of about 7.5 DEHP is expected to be strongly adsorbed to organic matter. In the environment DEHP therefore is expected to be found in the solid organic phase. Due to the high log Kow value and low water solubility, the equilibrium for DEHP will be in favour of particles. This is further enhanced by bonding (van der Waahls type bonds) between mineral surfaces and the benzene rings and carbonyl groups of the phthalate ester (ECETOC 1985). Thus the transport of DEHP in aquatic environments will to a high degree depend on the transport of particles. This also indicates that the mobility of DEHP in soil would be low, but since DEHP is adsorbed to for instance organic acids (especially humic substances) and to non-humic matter such as proteins, this may not always be the case. Adsorption to colloidal matter may also enhance subsurface transport of contaminants in soils through cracks and micropores. Experimentally derived Koc with fresh water confirm this with values between 63,100 and 888,000 l/kg (Howard 1989)(Sullivan et. al. 1982)(ABC-Laboratories 1991). The default value obtained from the EUSES model, 589,000 l/kg (based on log Kow = 7), falls within this range. The PCKOC model (Syracuse model, Meylan 1992) based on structure analysis estimates Koc to 165,000. This value seems to be more in agreement with the majority of experimental values than the EUSES estimate and is therefore used in the EUSES calculation.

There are indications that the adsorption of DEHP on particles is enhanced by the presence of salt). In a comparative laboratory study with salinity between fresh water and sea water the adsorbed amount increased 8-9 times when salinity increased from 0 to 29.5 promille (Al-Omran

and Preston 1987). This is consistent with reported decreased water solubility with increasing salinity (Howard 1985). Furthermore, Sullivan et. al. 1982 reported Koc-values from a marine sediment/water system of 794,000 to 1,260,000.

3.1.2.2.2 Precipitation and volatilisation

DEHP has a vapour pressure of $3.4 \cdot 10^{-5}$ Pa (20-25°C), which indicates a low evaporation rate from its pure state. The evaporation is also dependent on the nature of the matrix of the product. This was demonstrated in a laboratory study where 75% of DEHP applied on a glass plate was dissipated after 12 hours in the dark (temperature not reported, Furtmann 1996, p.39). The evaporation from a polymer matrix, however, is much lower < 1%/year (Berntsson 1984 in BUA 1986). Nevertheless, DEHP does evaporate from products. High concentrations are observed in indoor environment (see Section 4.1.1.2.2). The temperature probably is a key factor to understand these evaporation patterns. In the environment high peak temperature occur during sun light radiation. Up to 70°C has been reported from cars exposed to the sun (BUA 1986). The vapour pressure is 320 times higher at 70°C compared to 20°C (see Section 1.3.5). DEHP evaporated during high temperature peaks will probably condense at normal temperature and form small particles or adsorb to other particles.

Reliable studies on evaporation from different environmental compartments such as soil, vegetation etc. are not available.

A Henry's Law constant of 4.43 Pa · m^3 /mol ([390.6 · 0.000034] / 0.003, based on preferred data for 20°C) indicates a moderate evaporation from a pure water solution ("semi-volatile"). Klöpffer et. al. have measured an evaporation half-life of 139 days from water (0.35 mg/l, 22°C). In natural water and STP, however, adsorption to organic matter will probably reduce the evaporation potential significantly.

On the other hand, in a study on uptake in crop, plants were observed to assimilate DEHP that was probably evaporated from soil fertilised with STP sludge (see also Section 3.1.1.4.2). Foliar uptake of DEHP and similar chemicals evaporated from soil has been observed (Topp et. al. 1986, Fries 1981). ECPI have presented an estimation of the evaporation rate of DEHP from STP sludge spread on land. Based on European conditions the evaporation rate is roughly calculated to be at a maximum of 28 mg/m^2 · 1,000h at 20°C (ECPI 1997). This indicates that DEHP have a theoretical potential to evaporate from soil in considerable amounts. However, the real situation is probably more complex. To get a better understanding more knowledge of the influence of adsorption and daily temperature variations on the volatilisation of DEHP from soil is needed.

DEHP emitted to the atmosphere will, due to its low water solubility and vapour pressure, to some extent be adsorbed to particles.

In cold areas with temperatures below zero in the winter DEHP is expected to accumulate in snow and ice. High peak of DEHP may then be released into the melting water.

3.1.2.2.3 Distribution in wastewater treatment plants

The Simple Treat model is recommended in TGD to estimate the fate of a chemical in the STP. For this the EUSES 1.0 model was used.

Input data:

Biodegradation:	Ready biodegradability (see Section 3.1.2.1.2)
Vapour pressure:	3.4E-5 Pa (20°C)
Water solubility:	0.003 mg/L (see Section 0)
Molecular weight:	391
log Kow:	7.0 Comment: The log Kow is assumed to be about 7.5. However, the highest log Kow accepted in modelling is 7 in EUSES (due to lack of linearity in equations used).
Henrys Laws constant:	(3.4E-5 · 391) / 0.003 = 4.43 Pa m3 mol-1 →log H = 0.65

The results are presented in **Table 3.42**.

Table 3.42 Output data from EUSES

Removal in STP	
Adsorbed to sludge	78%
Released to water recipient	6.8%
Degraded in STP	15%
Evaporation to air	0.016%
Removal rate	93.2%

The calculation indicates that the overall removal of DEHP in a STP is approximately 93%. The major part is expected to be adsorbed to the sludge. Approximately 7% is expected to be released to the water recipient and 15% is expected to be degraded in the STP. Very little DEHP is expected to evaporate. In monitoring studies on STPs, removal rates above 90% are frequently reported (see Section 3.1.4.2). This confirms the removal rate obtained in these calculations.

3.1.2.3 Accumulation and metabolism

3.1.2.3.1 Aquatic organisms

Several bioaccumulation studies of variable quality are available. Most of them are excluded from this assessment due to various reasons, e.g. nominal test concentrations much higher than the solubility of DEHP in water, duration of exposure not sufficient to reach equilibrium, combined exposure with other chemicals, or static test conditions. Available studies are summarised in Table 9, Annex 1. Studies assessed to be of acceptable quality are reviewed below.

Bioaccumulation in fish

The bioaccumulation of ring-^{14}C-labelled DEHP and 32 other chemicals in bluegill sunfish (*Lepomis macrochirus*, mean fat 0.37 - 0.95 g) was studied in a flow-through system (Barrows et al, 1980). The fish were exposed to 5.82 ± 0.90 µg DEHP/l (mean measured) for 42 days,

followed by a depuration phase of 7 days. The resulting BCF for DEHP was 114, based on total ^{14}C-residues. The elimination half-life was reported to be 3 days.

The bioaccumulation of carbonyl-^{14}C-labelled DEHP in adult fathead minnows (*Pimephales promelas*) was investigated in a flow-through study (Mayer and Sanders, 1973). The fish were exposed to a test concentration of 1.9 µg DEHP/l for 56 days at 25°C, followed by a depuration phase of 28 days. Equilibrium concentration in fish tissues was reached after 28 days of exposure, and the BCF was determined to be 1380. Elimination half-life was 7 days.

The bioaccumulation of carbonyl-^{14}C-labelled DEHP in adult fathead minnows (*Pimephales promelas*, mean WWT 1.24 ± 0.31 g) was investigated in a flow-through study (Mehrle and Mayer, 1976; Mayer, 1976). Test concentrations of DEHP were 1.9, 2.5, 4.6, 8.1, 14, 30 and 62 µg/l (mean measured). The fish were exposed to DEHP for 56 days at 25°C, followed by a depuration phase of 28 days.

Besides the parent compound, the major metabolite found in the fish tissues was MEHP, accounting for 12 - 50% of the recovered residues after the exposure period. The proportion of MEHP increased with the exposure concentration. The data presented in **Table 3.43** below represent the level of residues in fish after 56 days of exposure in a flow-through study. The composition of residues in water was not reported in the study, since the concentrations were measured radiometrically only.

Table 3.43 Residue composition in fish after 56 days

	Residue composition in fish after 56 days (% of total recovered).					
Conc. in water (µg/l)	DEHP	MEHP	Phthalic acid	MEHP conjugate	Phthalic acid conjugate	Other
1.9	79	12	2.5	4.1	1.2	1.2
2.5	70	24	1.7	2.2	1.1	1.0
4.6	69	23	4.1	1.2	0.6	2.1
8.1	70	21	5.4	1.0	1.2	1.4
14	60	30	3.6	0.6	5.1	0.7
30	42	40	11	0.4	6.6	0.3
62	33	50	5.7	0.4	9.8	1.1

Bioaccumulation factors based on total residues, DEHP+MEHP, and DEHP in fish, respectively and exposure concentrations in the water given as total ^{14}C are given in **Table 3.44** below.

Table 3.44 Bioconcentration factors for fish after 56 days of exposure

Bioconcentration factors after 56 days.			
Mean exposure conc. (µg/l)	BCF total 14C	BCF DEHP + MEHP	BCF DEHP
1.9	737	670	582
2.5	880	827	616
4.6	891	820	614
8.1	444	404	311

Table 3.44 continued overleaf

Table 3.44 continued Bioconcentration factors for fish after 56 days of exposure

Bioconcentration factors after 56 days.			
Mean exposure conc. (µg/l)	**BCF total 14C**	**BCF DEHP + MEHP**	**BCF DEHP**
14	357	321	214
30	287	235	121
62	155	129	51

At concentrations higher than approximately 5 µg/l, BCF was inversely correlated with the test concentrations. However, in the two highest test concentrations, steady state does not seem to have been reached during the 56 days of exposure. At the lowest three test concentrations, which were below or close to the solubility of the test substance (1.9, 2.5 and 4.6 µg/l), BCF were also calculated using a computer program BIOFAC (U.S. EPA, 1982), resulting in a mean BCF of 842±105 based on total ^{14}C. This value will be used in the further assessment of DEHP.

The mean depuration half-life was 12.2 days based on DEHP, 13.6 days based on total ^{14}C-residues. At the lowest three concentrations in water, the half-life was calculated (BIOFAC) to be 7.6±0.73 days. The bioaccumulation of carbonyl-^{14}C-labelled DEHP was also investigated in early life stages of rainbow trout (*Salmo gairdneri*) in a flow-through study (Mehrle and Mayer, 1976). Test concentrations of DEHP were 5 - 54 µg/l (nominal). Rainbow trout eggs were exposed for 12 days at 10°C prior to hatch, and the resulting fry were exposed for an additional period of 90 days. No depuration period was reported.

The bioaccumulation factors in the swim-up fry (24 days after hatch) of *Salmo gairdneri* were 42-113, based on parent compound and nominal test concentrations in the water. Of the recovered ^{14}C-residues in fry, 40 - 60% was accounted for by glucuronid conjugates, 7 - 15% by phthalic acid and 15 - 20% by MEHP. Since the calculations were based on nominal test concentrations, the BCF values can be assumed to be underestimated.

The bioaccumulation of carbonyl-^{14}C-labelled DEHP in estuarine mullet (*Mugil cephalus*, mean WWT 4.3±0.4 g) was investigated in a flow-through study with artificial seawater (Park et al, 1990). The fish were exposed to DEHP at a nominal concentration of 50 µg/l for 15 days at 25°C, followed by a depuration phase of 14 days. Equilibrium was reached by the first day of exposure, after which the bioconcentration factor remained between 220 and 270, based on measured total ^{14}C. Elimination half-life was determined to be 1.8 days.

In a recent study (Scholz et al, 1998), the bioaccumulation of ring-deuterated DEHP in *Cyprinus carpio* was investigated. Fish were exposed to nominal concentrations of 5 (dissolved with 72 µg/l acetone), 50 and 150 (dissolved with 300µg/l MARLOWET R 40) µg/l for 42 days, followed by 14 days of depuration in a flow-through system (50 l/hour). Residues in fish and water were analysed by GC/MS.

In the two higher test concentrations, equilibrium was not reached – BCF was still increasing at the end of the exposure period. The highest BCF values were 34 and 20 based on mean measured exposure concentrations of 48 and 144 µg/l, respectively. Related to fat content of the fish, the corresponding BCF values were calculated to 1,069 and 459. At the lower exposure concentration (nominal 5 µg/l), measured levels in water were fluctuating between 0.6 and 6.7 µg/l, with a mean of 3.0 µg/l. The highest BCF, 221 (based on mean measured concentrations), was observed after ca four weeks of exposure. Elimination half-life during the depuration period was 1-2 weeks.

Caunters et al (2004) calculated bioaccumulation factors (BCF) and biomagnification factors (BMF) as a part of their multigeneration toxicity study on Fathead minnow (*Pimephales promelas*). The study is reviewed in detail in Section 3.2.1.1. The fish were exposed to DEHP both via water and via the food. Two treatments, Low Dose Food (LDF) and High Dose Food (HDF) were employed, both having the same nominal DEHP concentration in the water (5.0µg/l) but differing with respect to the concentration in the food, 125 mg/kg and 500 mg/kg, respectively The mean measured DEHP concentration in water was 3.2µg/l in the the LDF-group and 2.6µg/l in the HDF-group. The mean measured DEHP concentration in the food was 115 mg/kg in the LDF-treatment and 474 mg/kg in the HDF treatment

Calculated Bioconcentration factors for DEHP based on the assumption that the uptake was from the water only ranged between 202 and 785 for the different groups. When including MEHP the BCF:s ranged from 217 to 825 (see **Table 3.69**).

Calculating BMF factors with the assumption that all uptake was from the food gave BMF factors ranging between 0.01 and 0.06 for the different groups (see **Table 3.70**). When lipid normalised the BMF factors ranged from 0.01 to 0.07.

In a toxicity study, reviewed in detail in Section 3.2.1.1, Norman et al. fed Atlantic salmon with DEHP contaminated food with nominal concentrations of 0, 400, 800 and 1,500 mg/kg food (dwt). The mean measured concentrations, based on measurements at the start and the end of the exposure period, was 358, 827 and 1648 mg/kg for the three exposure levels, respectively. Feeding with DEHP contaminated food was initiated at the end of the yolk sac stage and continued for 4 weeks.

DEHP and MEHP concentrations in the exposed fish were measured at the termination of the uptake period. The concentration of DEHP was 0.66, 1.3 and 2.6 mg/kg wet weight in the three exposure levels, respectively. The correspondeing MEHP concentrations were 0.22, 0.50 and 0.76 mg/kg wet weight. Based on this and the measured exposure concentrations lipid normalized BMF factors were calculated. The BMF factors for DEHP was 0.011, 0.0064, 0.0073 for the three exposure levels, respectively.

Mackintosh et al. (2004) investigated the distribution of phthalate esters in a marine aquatic food web in order to study their potential for biomagnification. This was done by sampling of 18 species representing approximately 4 trophic levels in False creek harbour, Vancouver. The species were chosen to represent both benthic and pelagic based food webs and a variety of feeding strategies, sizes and life histories. For each species 9 individual samples were taken. The samples were collected between June and September at three different locations in False creek harbour. Samples were analysed for 13 different phthalates including DEHP. The samples were also analysed for six congeners of PCB. Only the results for DEHP will be accounted for in detail in this summary. There were no statistically significant differences in contamination levels in the samples from the three different locations.

The species were assigned to trophic levels using both a trophic position model and stable nitrogen isotope analysis. For each substance the lipid normalised log concentration in the different species were plotted against their trophic position or $\delta^{15}N$ ratio. Food-web magnification factors (FWMFs) were determined as the antilog of the slope of the log-linear regression between substance concentration and trophic level or $\delta^{15}N$.

The FWMF represents the average increase in lipid equivalent chemical concentration for a 1.0 unit increase in trophic position or a 3.4% increase in stable nitrogen isotope ratio ($\delta^{15}N$). For example, a FWMF of 2 means that the lipid equivalent concentration increases 2-fold for a 1-step increase in trophic position or an increase by 3.4% of $\delta^{15}N$. Conversely, a FWMF of 0.25

indicates that the concentration at a given trophic level is 25% of the concentration at the previous trophic level. Thus, a FWMF greater than 1 indicates biomagnification in the food web whereas a FWMF less than 1 indicates trophic dilution.

For the low molecular weight phatalates there were no statistically significant relationships between lipid equivalent concentrations and trophic position. For the high molecular weight phthalates the lipid equivalent concentrations significantly declined with increasing trophic position. The FWMF for DEHP was 0.32 (0.14-0.17, 95% CI) based on trophic position and 0.34 (0.18-0.64, 95% CI) based on $\delta^{15}N$ indicating that DEHP does not biomagnify in the aquatic food web studied, rather it undergoes trophic dilution. For comparison the FWMFs for the 6 PCB congeners ranged from 1.8 to 9.5 based on trophic position and from 2.05 to 6.98 based on $\delta^{15}N$.

Bioaccumulation in aquatic invertebrates

The bioaccumulation of ^{14}C-ring-labelled DEHP in third instars larvae of *Chironomus plumosus* was investigated in a flow-through study (Streufert et al, 1980). The larvae were exposed to a test concentration of 0.2 ± 0.02 µg/l (measured) for 9 days at 22 ± 1°C. Equilibrium was reached after 7 days, resulting in a BCF of 408. Half of the larvae were withdrawn after 4 days of exposure and transferred to clean water for 4 days of depuration. The elimination half-life was reported to be 3.4 days. In parallel studies, the effects on bioaccumulation of sand and hydrosoil substrate were investigated. The presence of adsorptive substrates was shown to reduce the concentrations of DEHP in solution and also the residue levels in the organisms.

The bioaccumulation of ^{14}C-labelled DEHP in the aquatic invertebrates *Gammarus pseudolimnaeus*, *Chironomus plumosus, Daphnia magna, Hexagenia bilineata* and *Asellus brevicaudus* was investigated in a flow-through study (Sanders, Mayer and Walsh, 1973).

Chironomus plumosus, Daphnia magna and *Hexagenia bilineata* were exposed to DEHP at measured concentrations of 0.3 or 0.1 µg/l for 7 days. *Gammarus pseudolimnaeus* was exposed to two concentrations, 0.1 and 62.8 µg/l, for 14 and 21 days, respectively. *Asellus brevicaudus* was exposed to 1.9 and 62.3 µg DEHP/l for 21 days at both concentrations. The organisms were not fed during the experiments.

Steady state was reached after 7-21 days of exposure for *Gammarus* and *Asellus* sp. For *Gammarus*, BCF was 13,400 and 260 at the low and high test concentrations, respectively, calculated on a dry weight basis. Assuming a water content of 80%, the corresponding values based on fresh weight are 2,680 and 52, respectively. *Asellus*, exposed to 1.9 and 62.3 µg/l, showed steady state concentrations 70 and 250 times that in the water, or 14 and 50 times when calculated on a fresh weight basis in the two exposure concentrations respectively. *Chironomus*, *Daphnia* and *Hexagenia* sp. did not reach steady state concentrations within the 7 days exposure periods. The highest measured concentrations in these organisms based on dry weights were 3,100, 5,200 and 2,300 times that in the water, corresponding to 620, 1,040 and 460, respectively, based on fresh weight. The elimination half-life of ^{14}C in *Gammarus* was reported to be less than 4 days.

Some of the results from Sanders, Mayer and Walsh, 1973 are also reported in Mayer and Sanders, 1973. In this report the results appear to be given based on wet weights and the BCFs reported for *Gammarus* and *Chironomus* at the low dose exposure were 3,600 and 350, respectively. The BCFs reported for *Daphnia* and *Hexagenia* were 420 and 575, respectively.

The bioaccumulation of carbonyl-^{14}C-labelled DEHP in *Daphnia magna* was investigated in a semi-static test - renewal of test medium three times a week (Brown and Thomson, 1982).

Nominal test concentrations were 3.2, 10, 32 and 100 µg/l. The daphnids were exposed for a period of 21 days at 20°C. Concentrations of DEHP were measured before and after each renewal of test medium. Concentrations in the daphnids were measured only at the end of the exposure period.

Bioconcentration factors based on nominal ^{14}C-activity ranged between 140 and 268 with a mean BCF of 209. However, the exposure concentrations were shown to decrease by 40-60% between each renewal of test medium. BCF based on the mean of concentrations measured in renewed and "old" test medium ranged between 190 and 330, mean 268 (evaluator's calculation). The elimination rate was not investigated.

The bioaccumulation of ring-^{14}C-labelled DEHP in *Mytilus edulis* was investigated in a flow-through study (Brown and Thomson, 1982). Mean measured test concentrations were 4.1 and 42.1 µg/l. The mussels were exposed for a period of 28 days at 15°C, followed by 14 days of depuration. Bioconcentration factors based on mean measured ^{14}C-activity were calculated to be 2,366 at the lower test concentration, 2,627 at the higher concentration - mean 2,497. The elimination half-life was determined to be 3.5 days. These BCFs may overestimate the bioaccumulation potential of DEHP in mussels since they are based on^{14}C-activity and not on DEHP and metabolites (i.e. MEHP). Some of the radioactivity measured in the mussels may be due to e.g. $^{14}CO_2$ assimilated by algae and subsequently ingested by the mussels.

The bioaccumulation of ^{14}C-labelled DEHP in a number of marine aquatic species was studied in a semi-static mesocosm study (Perez et al, 1983). The microcosms were 140 litre glass tanks containing seawater and a benthic box core, both collected from the natural system being simulated. Water turnover was simulated by removal and replacement of 10 litres of water per tank three times a week. Nominal test concentrations were 1, 10 and 100 µg/l. The microcosms were exposed for a period of 30 days at 18°C (summer scenario) and 1°C (winter scenario), respectively.

DEHP concentrations in the water decreased exponentially throughout the study. The mean measured concentrations in the water were 0.58, 5.8 and 59 µg/l for the winter situation and 0.18, 1.2 and 16 µg/l for the summer situation. In the sediment, mean measured concentrations were 41, 229 and 4786 µg/kg dwt in the winter, 43, 219 and 6166 µg/kg dwt in the summer scenario. Bioconcentration factors for the filter feeder bivalves *Pitar morrhuana* and *Mulinia lateralis*, calculated on the basis of the mean measured DEHP concentration in water, were 436-1,381 and 2,456 – 3,891, respectively, during the winter scenario. The corresponding values for the summer scenario were 364 – 1,124 and 932 – 3,311. For the zooplankton *Acartia sp.*, winter BCFs ranged between 169 and 2,572, summer BCFs between 1,995 and 5,376. Whether these BCFs are based on the total ^{14}C activity in the organisms or the concentration of DEHP is unclear from the report. The highest BCF values for Acartia were observed at the lowest test concentrations (0.58 µg/l for the winter situation, 0.18 µg/l for the summer situation). The lowest summer BCF, 1995, was measured at a DEHP concentration in water of 16 µg/l. For the benthic polychaete *Nephtys incisa* and the benthic bivalve *Nucula annulata*, the calculated BCFs were based on mean measured concentrations of DEHP in sediment. None of these values were higher than 10. Due to the fact that the DEHP concentration in the water decreased exponentially throughout the study the results from this study will not be used in the risk characterisation.

Conclusions

The results of the studies show that the bioaccumulation of DEHP varies between different aquatic species. In fish the bioconcentration factors, based on total radioactivity, range between 114 and 1,380. Equilibrium time vary between one and > 56 days. The studies are performed

with ^{14}C-labelled DEHP. The measured radioactivity refers to total ^{14}C-residues, and the concentration of DEHP may be overestimated. However, since the main metabolisation product of DEHP is the reprotoxic MEHP these data are assumed to be valid. In one study, the metabolisation products are quantified. Calculations based on DEHP+MEHP result in BCF between 129 and 827. Furthermore, the BCF:s calculated in the multigeneration toxicity study on fathead minnow gave BCF-values for DEHP ranging from 202 to 785 for the different groups. When including MEHP the BCF:s ranged from 217 to 825.

The uptake in fish via food seems to be limited based on the low BMF-factors obtained in the studies by Norman et al. and Caunters et al. (2004). DEHP does not seem to biomagnify in the food chain as indicated by the results of the study by Mackintosh et al (2004) where there was a negative correlation between DEHP concentrations and trophic level.

In general, the bioaccumulation of DEHP decreases at concentrations higher than approximately 5 µg/l, and the proportion of MEHP found in fish tissues increases with the exposure concentration. This could be due to a more efficient metabolism at higher exposure levels. Another possible explanation is that at test concentrations above the non-colloidal water solubility (approximately 3 µg/l), a significant amount of DEHP is in the colloidal form, which could make it less bioavailable. Decreasing BCF with increasing concentrations of DEHP in water was also observed for zooplankton.

The elimination half-life of DEHP, based on total radioactivity, is between 3 and 14 days in the different fish species tested. The major degradation pathway seems to be through hydrolysis of the ester group resulting in the monoester MEHP and subsequent glucuronid conjugation.

Since DEHP is readily adsorbed onto organic surfaces and particles in the water phase and to sediment, the highest bioaccumulation factors are obtained for zooplankton (Acartia sp.) with a high surface/weight ratio, for Gammarus sp., a sediment dwelling amphipod, and for filtrating molluscs. For these kinds of organisms, DEHP in the colloidal form and DEHP adsorbed to particles can be assumed to be more easily available.

In the standard scenario on secondary poisoning a fish eating animal is selected. Since fish show relatively low BCF values compared to invertebrates, invertebrate eating animals are probably a more critical target group. In the Baltic Sea, for example, the filter-feeding mussel Mytilus edulis is the predominant food for birds such as the long tailed duck (*Clangula hyemalis*) and the common eider (*Somateria mollissima*) (Gilek et. al. 1997). Therefore, besides the fish scenario two invertebrate scenarios are introduced. The following BCF values are chosen to represent realistic worst case conditions:

Type of prey	**BCF***	**Reference**
Fish	840	(Mayer et. al., 1976)
Invertebrate, mussels	2,500 WWT	(Brown and Thomson, 1982)
Invertebrates, amphipods	2,700 WWT	(Sanders, Mayer and Walsh, 1973)

* based on C-14 technique

3.1.2.3.2 Terrestrial organisms

Bioaccumulation in earthworms

Earthworms (*Eisenia foetida foetida*) were exposed to soil with a nominal DEHP concentration of 1,000 mg/kg (dwt) for 14 days in a toxicity test (Diefenbach, 1998b, see Section 3.2.3.2). After the exposure period the earthworms were moved to uncontaminated soil for a depuration period of 8 days. The earthworms were analysed for DEHP after the 14 days exposure period and after further 1, 4 or 8 days in uncontaminated soil. The derived dry weight BCFs based on the nominal DEHP concentration in soil were 0.126 (after 14 days of exposure), 0.174 (after 1 day of depuration), 0.211 (after 4 days of depuration) and 0.099 (after 8 days of depuration). Assuming a typical dry to wet weight conversion factor of 0.15 for earthworms and of 0.88 for soil, a BCF of 0.034 based on wet weights can be derived from the highest dry weight BCF of 0.21.

The test protocol does not say anything about the number of earthworms analysed or whether or not the gut content of the worms was removed before analysis. The BCFs are calculated based on the nominal concentration of DEHP in soil. The DEHP concentration in the unexposed control worms was 21 mg/kg dwt and 47 mg/kg dwt in two replicate samples. This may indicate uptake of DEHP from the control soil or problems with contamination in the analytical procedure. Furthermore, it is probable that steady state was not reached in this study since the exposure period was only 14 days Taken together this makes the BCF-values derived in this study unsuitable for use in the risk characterisation.

Albro et al. (1992) studied the metabolism of DEHP in earthworms (*Lumbricus terrestris*). Individual worms were given [7-^{14}C]-DEHP by oral gavage. Three different doses of DEHP were tested, 35.8, 179.2 or 358.5. Analysis was made 1, 2 and 7 days after administration for the lowest dose group and 7 days after administration for the middle and high dose group. The distribution of ^{14}C is given **Table 3.45** below.

Table 3.45 Distribution of residues in earthworms

Dose (µg/worm)	Time (days)	DEHP in worms (% of initial radioactivity)	MEHP in worms (% of initial radioactivity)	Other metabolites (% of initial radioactivity)	Total 14C in worms (% of initial radioactivity)	14CO2 (%of initial radioactivity)	14C in bedding (% of initial radioactivity)
35.8	1	66.8	11.6	11.6	89.9	0.5	9.6
35.8	2	75.2	10.5	9.1	94.8	1.7	3.5
35.8	7	67.4	4.8	1.3	73.5	24.8	1.7
179.2	7	65.3	4.8	1.0	71.1	25.0	3.9
358.2	7	67.4	5.2	1.1	73.7	24.9	1.4

One day after administration of the lowest dose, approximately 90% of the initial radioactivity was associated with the worm. Of this 67% was DEHP and 12% was MEHP. Seven days after administration DEHP still accounted for 67% of the initial radioactivity while MEHP had decreased to approximately 5% of initial radioactivity. The middle and high dose showed the same pattern. This indicates that DEHP is initially hydrolysed to MEHP by the worm and that DEHP is translocated within the worm to sites with low metabolic capacity leading to a slow overall metabolism and thus elimination of DEHP. Based on the elimination of total radioactivity from the worms in the Albro study Staples et al. (1997) calculates a first order depuration rate of 0.04d^{-1} for *Lumbricus terrestris*. This depuration rate may not be a relevant measure of the elimination of DEHP from worms. The reason for this is that the elimination in the Albro study

does not seem to follow first order kinetics. After a rapid transformation to MEHP the DEHP concentration in the worms remains constant from day 1 after administration to the end of the study, day 7 (see **Table 3.45**).

In summary it can be concluded that no valid bioaccumulation study with earthworms is available. The study by Albro et al. shows that the metabolism of DEHP by earthworm is slow indicating that DEHP has a potential to accumulate in earthworms. EUSES calculates a BCF worm of 43 kg/kg. As there is a difference of about three orders of magnitude between the measured and the estimated BCF-value, it appears that the higher value is an overestimation. In a first approach, based on the experimental results and the low measured depuration rate, a reasonable worst case BCF of 1 will be used in the risk assessment.

Bioaccumulation in terrestrial plants

Several studies are available on bioaccumulation in terrestrial plants (Overcash et.al. 1986, Schmitzer et. al. 1988, Aranda et. al. 1988, Kirchmann and Tengsved 1991).

Overcash et al. (1986) studied uptake of DEHP and Di-n-octylphthalate (DOP) in fescue, corn, soybeans and wheat under greenhouse conditions. Plants were grown at different substance concentrations ranging between 0.044 and 4.4 ppm for DEHP respectively 0.022 and 2.2 ppm for DOP. The uptake was monitored by measuring ^{14}C in the plants, assuming that the ^{14}C detected is the parent compound. The highest uptake was recorded with fescue and corn harvested respectively 34 and 17 days after planting while lower uptake were observed in mature wheat and soybeans (see **Table 3.46**). The final soil concentration of DEHP was on average 25% if the initial applied concentration could be used to derive a BCF. The highest uptakes were recorded for the highest soil concentrations. Based on dry weights and initial or final soil concentrations, the accumulation with DEHP was:

Table 3.46 Bioconcentration factors day 17 and day 34 in different crops

Plant	Initial soil conc (mg/kg dwt)	Final plant conc. (mg/kg dwt)	BCF (17 days)	Final soil conc. (mg/kg dwt)	Average soil conc.* (mg/kg dwt)	BCF (34 days)
fescue	0.044	0.028	0.63	ca. 0.011	ca. 0.022	1.3
fescue	0.44	0.27	0.61	ca. 0.11	ca. 0.22	1.2
fescue	4.4	3.2	0.72	ca.1.1	ca. 2.2	1.4
corn	0.044	0.009	0.20	ca. 0.011	ca. 0.022	0.4
corn	0.44	0.022	0.05	ca. 0.11	ca. 0.22	0.1
corn	4.4	4.6	1.04	ca.1.1	ca. 2.2	2.1
soybean	0.044	0.0	0	ca. 0.011	ca. 0.022	0
soybean	0.44	0.012	0.027	ca. 0.11	ca. 0.22	0.05
soybean	4.4	0.011	0.0025	ca.1.1	ca. 2.2	0.005
wheat	0.044	0.0046	0.10	ca. 0.011	ca. 0.022	0.21
wheat	0.44	0.030	0.07	ca. 0.11	ca. 0.22	0.14
wheat	4.4	0.315	0.07	ca.1.1	ca. 2.2	0.14

* Geometric mean

The uptake for DOP was approximately 1 to 2 orders of magnitude lower.

Aranda et al. (1989) also tested the uptake of DEHP under greenhouse conditions in lettuce, carrot, chilli pepper and fescue using ^{14}C labelled DEHP. 4 soil treatments with initial DEHP concentration in soil between 2.65 and 14.0 mg/kg dwt was used. Based on dry weight, the average BCFs for lettuce, carrot top, root, chilli plant, chilli fruit and fescue were 0.47, 0.28, 0.13, 0.15, 0.08 and 0.24 respectively.

Schmitzer et al. (1988) studied the uptake of DEHP in barley and potatoes in outdoor experiments. As DEHP had completely disappeared from soil in the barley experiment, the results could not be used to derive a BCF. The initial and final soil concentrations were 1 mg/kg dwt and 0.033 mg/kg dwt respectively, based on ^{14}C measurements. The vegetation period was 111 days. For assessment purposes, an average concentration (geometric mean) of 0.18 mg/kg dwt can be used for the BCF estimation, as shown in **Table 3.47**.

Table 3.47 Concentrations of DEHP and bioconcentration factors in different parts of potato

Sample	Average soil conc. (mg/kg dwt)	Plant conc. (mg/kg fresh w.)	Plant conc. (mg/kg dwt.)*	BCF
potatoes, peeled	0.18	0.077	1.08	6.0
peel	0.18	0.032	0.45	2.5
shoots	0.18	0.119	1.67	9.2
roots	0.18	0.160	2.24	12.4
plant total	0.18	0.076	1.06	5.9

* Calculated based on TGD defaults

These results are also based on ^{14}C measurements, assuming that the ^{14}C detected is the parent compound.

Kirchmann and Tengsved (1991) measured the concentration of DEHP in barley grains grown on sludge-amended soil. The application rate of sludge was 5 tones dwt/ha. The DEHP concentration in sludge was 116 mg/kg dwt, and the resulting concentration in grains was 530 µg/kg dwt. The DEHP found in the grain amounted to 0.22% of the dose from the sludge. The barley grown on N-fertiliser or pig slurry amended soil also contained DEHP at concentrations of 89-110 µg/kg, while no DEHP was detected in the fertiliser. The experimental area was not exposed for sludge before the experiment. This would indicate that a large portion of the DEHP in grains is due to uptake via the air (direct uptake and/or via the soil).

Discussion

Due to its high affinity to organic matter only a limited bioaccumulation of DEHP in plants is expected. The experimental studies confirm this with BCF ranging between 0.01 and 5.9. The highest BCF where observed on corn and potatoes. Lower BCF values where obtained for lettuce, carrot (top), chilli plant, soybeans and wheat.

The study on potatoes (Schmitzer et al. 1988) shows similar BCF in the whole plant. This indicates that DEHP was easily distributed from the root to the shoot. Since BCF in this case is based on ^{14}C the relatively high BCF in shoots may be a result of a transport of degradation products.

The results from Kirchmann and Tengsved (1991) indicate: (i) That DEHP in STP sludge will to some extent be distributed to the crop: (ii) A considerable part of the uptake of DEHP occurs from the background. (iii) DEHP will be transported within the plant (it is found in the

unexposed grain). The root uptake and distribution within the plant normally decreases considerably with increased hydrofobicity (according to TGD). An alternative uptake route into the plants is via the leaves. It is not clear if the particle bound phase or the vapour phase in the air is the main source for this uptake. Studies of other semi-volatile hydrocarbons indicate that the vapour phase may be an important uptake route (Welsch-Pausch et. al. 1995, McLachlan 1996, Kömp et. al. 1997, Kylin 1997). The results in this study may therefore be due to an evaporation of DEHP from the sludge.

The EUSES model calculates separate BCF for roots and leaves. The BCF was calculated to 275 in plant roots and 7.6 in plant leaves. The model assumes that most of DEHP is physically adsorbed to the root and only to a minor part transported to the leaves (based on Koc 165,000 and Log Kow, 7.0/ highest recommended). A comparison with experimental results indicates that this calculation show an agreement with the BCF for the leaves and an overestimation of the BCF for the root. The experimental derived results a rather uneven. It is therefore difficult to select a single value for the model. The highest value of 12 will therefore be used. Considering that the results are based on 14C-distribution this value still overestimates the real BCF, as metabolisation is not taken into account.

Using Kp_soil and an average bulk density of plant tissue RHOplant of 0.7 kg/l, a $K_{plant\text{-}water}$ value can be estimated:

$$K_{plant\text{-}water} = BCF \cdot RHOplant \cdot Cfdry\_wet \cdot Kp\_soil = 12 \cdot 0.7 \cdot 0.07 \cdot 3{,}300 = 1{,}940\ m^3/m^3$$

With 'Cfdry_wet' being the plant dry to wet conversion factor.

This value will be used for the indirect exposure of humans via the environment.

3.1.3 Calculation of Predicted Environmental concentrations (PECs)

The local PECs for life cycle stage 1 production, were calculated manually mainly based on site specific information.

The generic local PECs for the different polymer processing techniques (life cycle stage 2) were also calculated manually but based on TGD default emissions. Local site-specific releases to water have been calculated using measured data and measured (where available), or default (from Emission Scenario Document) releases to air, see sections 3.1.1.2.1, and 3.1.1.2.2. The reported emissions to water (measured after waste water treatment) were re-calculated to levels before STP (using EUSES default efficicency of STP) and then modelled as passing through the STP when calculating PECs in the EUSES model. This was to make sure that any DEHP in STP-sludge is taken into account when calculating concentrations in food. As no data on inflow, concentration in inflow to STP etc was reported, PEC for STP was not calculated for the site-specific scenarios. For the site-specific scenarios, emission days were set to 300, and dilution was set to default from TGD, since no actual values were reported.

The EUSES model was used to calculate local predicted environmental concentrations (PEC) for the remaining 10 life cycle stages: 3a-b, 4a-b, 5a, 6, 7a, and 8a-c (see **Table 3.48**). An overview of the EUSES files used for calculating PECs and human exposure via the environment is given in Annex 3.

Life cycle stages 1b, 7b, and 8d-e are regarded as diffuse sources and are only used for their contribution to regional/continental PECs. The regional/continental emissions from life cycle

stages 1a and 2 are introduced in the EUSES calculation in use pattern 1 prod and 3 proc, respectively.

Table 3.48 Overview of relevant scenarios for PEC calculation for DEHP

Life cycle stage	Description		PECLocal		EUSES Use pattern
			Site specific	Generic	
1a	Raw material	Production	X		1 prod.
1b	Raw material	Transportation[1]		[1]	2 prod.
2	Polymer product	All Formulation and/or Processing		X	3 proc.
2a		Calendering	X	X	
2b		Extrusion-compound	X	X	
2c		Extrusion-products	X	X	
2d		Spread coating	X	X	
2e	Polymer product	Other plastisol + car undercoating		X	
2f		Injection moulding/extrusion		[2]	
2g		Various plastisol applications		[2]	
3a	Non-polymer:	Formulation		X	4 form.
3b	Sealants, adhesives	Processing/Application	X	X	4 proc.
4a	Non-polymer:	Formulation		X	5 form.
4b	Lacquers and paints	Processing/Application		X	5 proc.
5a	Non-polymer:	Formulation		X	6 form.
5b	Printing inks	Processing/Application		[2]	
6	Non-polymer: Ceramics	Formulation		X	7 form.
7a	End Product uses	Municipal STP (releases to waste water)		X	1 priv.
7b		Diffuse emissions [3] (releases to air, surface water, urban soil)		[1]	2 priv.
8a	Recovery	Paper recycling		X	6 recov.
8b	Recovery	Car shredding		X	8 recov.
8c	Waste	Incineration		X	9 recov.
8d	Waste	Municipal land fill		[1]	1 recov.
8e	Waste	Waste remaining in the environment		[1]	1 recov.

1) Regarded as diffuse sources. Local PECs not calculated
2) Local PEC not calculated. These emissions are added to life cycle stage 7a and b (see Section 3.1.1.2).
3) Emissions to air, surface water and urban soil from lifecyclestages 7, 2f, 2g and 5b.

Urban/Industrial soils

Urban/industrial soils are assumed to be the major target for the emissions of DEHP (77%, see **Table 3.38**). However, the EUSES model does not calculate any PEC/PNEC ratios for this

compartment. The model only calculates a regional PEC for urban/industrial soil. It can be assumed that environmental concentrations locally are higher e.g. along roadsides. In this assessment therefore the local PEC for urban/industrial soil is assumed to be > [PECurban/ind. soil].

3.1.3.1 Local PECs

For production sites local PECs for all individual sites are calculated separately based mainly on site-specific data. For polymer formulation/processing generic local PECs for the different types of processes are calculated separately. The site-specific PECs are calculated using the EUSES model (v 1.0). The generic local PECs for the remaining scenarios are calculated with the EUSES model (v 1.0).

For the calculation of local PECs for production and polymer formulation/processing the equations presented below were used:

3.1.3.1.1 Local PEC for STP

Daily release has been obtained by dividing the reported amounts of emission to water with the number of working days (300 days in continuous working systems). The concentration of DEHP in the influent of the STP is calculated with the following formulae:

Equation 1

Clocalinf = (Elocalwater · 106) / (EFFLUENTstp) = (Elocalwater · 106) / 2 · 106 = Elocalwater / 2 [mg/l]

Equation2

Clocaleff = Clocalinf · [1 - STPremoval] = Clocalinf · 0.068 [mg/l]

Equation 3:

a) PEClocalSTP = Clocaleff = Clocalinf · 0.068 [mg/l]

Explanation of symbols

STPremoval	Removal rate [in STP] = 0.932 (see 3.1.3.5)	[-]
$Clocal_{inf}$	Concentration in untreated water	[mg/l]
$Elocal_{water}$	Local emission rate to waste water during emission period	[kg/d]
EFFLUENTstp	$CAPACITY_{stp}$ · WASTEWinhab = 10,000 · 200=2,000,000	[l/d]

PEClocalSTP for production and polymer formulation/processing sites are presented in **Table 3.49** and **Table 3.51**. $PEClocal_{STP}$ has not been calculated for site-specific processing and formulation sites since no data on concentration in inflow, total flow etc has been reported and the risk characterisation shows very low risks.

3.1.3.1.2 Local PEC for surface water

The concentration of DEHP in the effluent ($Clocal_{eff}$) of a STP is calculated with equation 3.

For DEHP it is assumed that the elimination in a STP is 93.2% (according to SIMPLETREAT, see section 3.1.3.5). From the effluent concentration in the STP, the local concentration in the receiving surface water can be calculated with the equations:

Equation 4

$Clocal_{water}$ = 1,000 · $Clocal_{eff}$ / [(1 + Kp_{susp} · SUSP · 10^{-6}) · DILUTION]

= **($Clocal_{eff}$/DILUTION) · 802**[µg/l]

Explanation of symbols

Kp_{susp}	Foc_{susp} · Koc = 0.1 (kg/kg) · 165,000 (l/kg) = 16,500	[l/kg]
SUSP	Concentration of suspended matter in the river = 15 (default, TGD)	[mg/l]
Foc_{susp}	0.1 (default, TGD)	[mg/l]
Koc	165,000	[l/kg]
DILUTION	Dilution factor (default = 10)	[-]
$Clocal_{eff}$	Concentration in effluent water	[mg/l]

Equation 5

PE**$Clocal_{surface\ water}$** = **$Clocal_{surface\ water}$ + $PECregional_{surface\ water}$**

PEClocalsurface water for the production and polymer formulation/processing sites are presented in **Table 3.49** and **Table 3.51**.

3.1.3.1.3 Local PEC for sediment

The concentration of DEHP in the sediment is calculated with the formula:

Equation 6

$PEClocal_{sediment}$ = $PEClocal_{surface\ water}$ · (Ksusp-water / RHOsusp) · 1000 =

= $PEClocal_{water}$ · 4,130 / 1,150 = **$PEClocal_{water}$ · 3.59 [mg/kg wwt]** =

= $PEClocal_{water}$ · 3.59 · 2.6^{a} [mg/kg dwt] = **$PEClocal_{water}$ · 9.34 [mg/kg dwt]**

Explanation of symbols

a	Conversion factor for "wwt" to "dwt" (based on defaults in TGD)	[-]
Ksusp-water	Suspended matter-water partitioning coefficient TGD default =4,130 (Koc=165,000)	[m^3/m^3]
RHOsusp	Bulk density of suspended matter. TGD default = 1,150 (0.1*2500+0.9*1000)	[kg wwt/m^3]
$PEClocal_{water}$	During emission period	[mg/l]

PEClocalsediment for the production and polymer formulation/processing sites are presented in **Table 3.49** and **Table 3.51**.

3.1.3.1.4 Local PEC for air

The concentration in air at 100 m from a point source can be estimated with the formula:

Equation 7:

$PEClocal_{air}$ = $PECregional_{air}$ + $Clocal_{air}$ = $PECregional_{air}$ + $Elocal_{air}$ · $Cstd_{air}$ =

= $PECregional_{air}$ + ($Elocal_{air}$ · 2.78 · 10^{-4}) [mg/m^3]

Explanation of symbols

$PECregional_{air}$		[mg/m^3]
$Clocal_{air}$		[mg/m^3]
$Elocal_{air}$	Local (max.) emission rate to air	[kg/d]
$Cstd_{air}$	Standard concentration in air at source strength of 1 kg/d = 2.78 · 10^{-4}	[mg/m3]

Based on its vapour pressure and - 2 < log HENRY < 2, the annual deposition over a radius of 1000 m around the source can be estimated as:

Equation 8:

$DEPtotal_{ann} = (Elocal_{air} + Estp_{air}) \cdot (Fass_{aer} \cdot DEPstd_{aer} + (1-Fass_{aer}) \cdot DEPstd_{gas}) =$
$= (Elocal_{air} + Estp_{air}) \cdot (1 \cdot 10^{-2} + (1-1) \cdot 4 \cdot 10^{-4}) = (Elocal_{air} + Estp_{air}) \cdot 10^{-2} =$
$= [Elocal_{air} + (Elocal_{water} \cdot Fstp_{air})] \cdot 10^{-2} =$
$= 0.01 \cdot Elocal_{air} + 1.6 \cdot 10^{-6} \cdot Elocal_{water}$ [mg/m2/d]

Explanation of symbols

Estpair	Local indirect emission to air from the STP = $Elocal_{water} \cdot Fstp_{air}$ [kg/d]
$DEPstd_{gas}$	Deposition flux of gaseous compounds (-2<log HENRY<2) at source strength of 1 kg/d=4 · 10^{-4} [mg/m^2/d]
$Fass_{aer}$	Fraction of the chemical bound to aerosol = 1 (measurements at the workplace have shown that 100% of the substance is bound to the aerosol)
$DEPstd_{aer}$	Standard deposition flux of aerosol-bound compounds at a source strength of 1 kg/d = 10^{-2} [mg/m^2/d]
$Fstp_{air}$	Fraction of the emission to air from STP = 1.6 · 10^{-4} (=0.016%, from Table 3.42) [-]
$Elocal_{water}$	Local emission rate to waste water during emission period [kg/d]
$Elocal_{air}$	Local (max.) emission rate to air [kg/d]

PEClocalair for production and polymer formulation/processing sites are presented in **Table 3.49** and **Table 3.51**.

3.1.3.1.5 Local PEC for agricultural soil

The PEC values in agricultural soil for the different scenarios were calculated with the EUSES model. It is assumed that the sludge from STP at the production sites is not distributed to agricultural soil.

For the different types of processing sites the $Elocal_{water}$ ("local release to water" from **Table 3.51**) is used as input value (at "local emission to wastewater during episode").

3.1.3.1.6 Local PEC for secondary poisoning

The PEC oral-aquatic is calculated by multiplying the bioconcentration factor (BCF) with $PEC_{surface\ water}$. The BCF for fish used in this assessment is 840. However, it has been shown that the BCF for fish decreases with increasing DEHP concentrations in water when the water solubility is exceeded (see Section 3.1.4). Therefore, the water solubility 3 µg/l is used as a limit for the calculation of $PEC_{oral\ aquatic\ fish,}$ i.e. when a $PEC_{surface\ water}$ exceeds the water solubility the water solubility is used for calculating $PEC_{oral\ aquatic.}$ The same approach is used for the calculation of $PEC_{oral\ aquatic\ zooplankton}$ using a wet weight BCF of 2,700. This approach may

underestimate the concentration in biota in highly contaminated areas since it can be assumed that in such cases the absorption of DEHP from food becomes increasingly important. The highest measured concentration in fish from an extensive study in Austria (Pfannhauser et al. 1997, see section 3.1.6.5) was 2.6 mg/kg wwt. This value compares quite well with the concentration derived when multiplying BCF with water solubility (840 · 3.0 µg/l · 10^{-3} = 2.52 mg/kg wwt). Unfortunately the concentration of DEHP in water was not measured in the Austrian study. For $PEC_{oral\ aquatic\ mussels}$ however, the calculated $PEC_{surface\ water}$ is used. This is based on a study where no difference in BCF was seen at DEHP concentrations of 4.6 and 46 µg/l respectively (see Section 3.1.2.3). Furthermore, since mussels are filter feeders it is assumed that the non-dissolved and particle bound fractions are bioavailable. A summary of PEC oral-aquatic is presented in **Table 3.50**, **Table 3.53** and **Table 3.55**.

Local $PEC_{oral\ worm}$ is not calculated for the production sites since STP sludge from production sites is not used as fertiliser. Local $PEC_{oral\ worm}$ for life cycle stage 2 are summarised in **Table 3.51** and for life cycle stages 3-8 in **Table 3.54**.

The regional PECs are summarised in **Table 3.56**.

Table 3.49 Local PEC for air, STP, Surface water and Sediment at different Production sites. Measured values in italic and default values in bold

Site	Elocal air	PEClocal air[4]	Emission to waste water before STP Elocal[2]	C local influent	STP yes / no[1]	C local Effluent[5] PECstp	Effluent flow[1]	Receiving flow[1]	Dilution	C local surf.water (/dilution) *total*	PEClocal surf.water during emission[4]	PEClocal surf.water[4] annual average	PEClocal Sediment
	(kg/d)	(mg/m^3)	(kg/d)	(mg/l)		(mg/l)	(m^3/s)	(m^3/s)		(µg/l)	(µg/l)	(µg/l)	(mg/kg dwt)[6]
1	0.082	3.0E-05	1.18	0.592	yes	0.0402	?	?	*700*	0.046	2.2	2.2	21
2	0.088	3.2E-05	31.2	15.6	yes	*0.0319*	*0.77*	*18.4*	24	1.07	3.3	3.2	31
3	0	7.6E-06	500	250	yes	*0.005*	*4.69*	*734*	157	0.026	2.2	2.2	21
4	0.068	2.7E-05	0.0205	0.0103	yes	**0.00085**	**0.023**	*10*	**10**	0.068	2.3	2.3	21
5	2.849	8.0E-04	487	244	yes	*0.00008*	*0.005*	sea	**10**	0.0064	2.2	2.2	21
6	1.973	5.6E-04	83.6	*20.4*	no	*20.4*	*0.12*	*100*	833	19.6	22.0	22.0	204
7	0.068	2.7E-05	0.0049	0.00247	yes	0.00017	*0.06*	*16*	267	0.00050	2.2	2.2	21
8	0.014	1.1E-05	386	193	?	*0.1*	*0.01*	*2*	200	0.40	2.6	2.6	24
9	0.027	1.5E-05	337	168	yes	*0.012*	*0.028*	sea	1000	0.0096	2.2	2.2	21
10	0.003	8.4E-06	0	0	x	0				0	2.2	2.2	21
11	0.118	4.0E-05	5.79	2.9	yes	0.197	?	sea	**10**	15.8	18	9.6	168
12	0.467	1.4E-04	80	40	?	2.72	?	?	**10**	218.1	220.3	181.5	2058
Equation number[3]		7	1			2 and 3				4	5		6

1) ECPI (1998a).
2) Elocalwater = Local emission rate to waste water during emission period [kg/d]. From Table 3.37.
3) See section 3.1.5.1.
4) In calculating PEC_{local} the PECregional for surface water = 2.2 µg/l is added as a background level (see Table 3.56).
   In calculating PEC_{local} the PECregional for air =7.5 ng/m^3 is added as a background level (see Table 3.56).
5) The effluent discharge rate for STP was set to default (2*10^6 l/d).
6) Values with ">" are based on a calculated surface water concentration that is above the water solubility. This probably underestimates the concentration in the sediment.

Table 3.50 PEC in aquatic biota (secondary poisoning). Life cycle stage 1: production sites

Production site	PECwater annual average1	PEC oral-aquatic2 (mg/kg wwt)		
	(ug/l)	Fish3	Mussel3	Invertebrates.4
Site 1	2.2	1.8	5.6	6
Site 2	3.2	2.2	6.8	8
Site 3	2.2	1.8	5.5	6
Site 4	2.3	1.9	5.6	6
Site 5	2.1	1.8	5.4	6
Site 6	22	2.2	30	8
Site 7	2.2	1.8	5.5	6
Site 8	2.6	2.0	6.0	7
Site 9	2.	1.9	5.5	6
Site 10	2.2	1.8	5.5	6
Site 11	9.6	2.2	14.8	8
Site 12	181.5	2.2	230	8

1) Annual average. From Table 3.49
2) BCF: fish=840; mussel=2500; zooplankton=2700. The water solubility 3 µg/l is used as a limit for the calculation of PECoral aquatic fish and PECoral aquatic zooplankton, i.e. when PECsurface water exceeds the water solubility the water solubility is used for calculating PECoral aquatic. For PECoral aquatic mussels however, the calculated PECsurface water is used.
3) PECs based on the assumption that 50% of prey is sourced from the local environment and 50% from the regional environment, according to TGD.
4) Predator fish are assumed to be stationary and consume all prey locally. Thus, $PEC_{oral\ invertebrates}$ is calculated based only on PEClocal surface water.

Table 3.51 Local PECs for different types of polymer formulation/processing sites (life cycle stage 2).

	Unit	2a Calendering	2b Extrusion compound	2c Extrusion products	2d Plastisol Spread Coating			2e Other plastisol		
					Air cleaning			Air cleaning		
					average	*with*	*without*	*average*	*with*	*without*
Total release per emission day/site[1]	kg/d	6.6	2.5	2.5	8.6	3.0	25.5	5.6	1.6	13.6
Elocal - waste water	kg/d	3.3	1.3	1.3	4.3	1.5	12.8	2.8	0.8	6.8
Influent STP Clocalinf	mg/l	1.65	0.6	0.6	2.2	0.8	6.4	1.4	0.4	3.4
Effluent STP Clocaleff[5]	mg/l	0.112	0.043	0.043	0.147	0.051	0.43	0.095	0.027	0.23
PEClocal – STP	mg/l	**0.112**	**0.043**	**0.043**	**0.147**	**0.051**	**0.43**	**0.095**	**0.027**	**0.23**
PEC agr.soil "30d" (fr. EUSES)	mg/kg dwt	**9.2**	**3.5**	**3.5**	**12**	**4.2**	**35.4**	**7.9**	**2.3**	**19.0**
PEC oral worm (fr. EUSES)	mg/kg wwt	**3.5**	**1.3**	**1.3**	**4.5**	**1.6**	**13.3**	**3.0**	**0.9**	**7.1**
Clocal surface water	µg/l	9	3.4	3.4	**11.8**	**4.1**	**34.8**	7.7	2.2	18.6
PEC surface water during emission[2]	µg/l	**11**	**5.6**	**5.6**	**14**	**6.3**	**37**	**9.9**	**4.4**	**20.8**
PEC surface water/annual average[2]	µg/l	**9.7**	**5**	**5**	**12**	**5.6**	**30.8**	**8.5**	**4**	**17.5**
PEClocal – sediment[4]	mg/kg dwt	**>105**	**>52**	**>52**	**>130**	**>59**	**>345**	**>92**	**>41**	**>194**
Elocal - air	kg/d	3.3	1.3	1.3	4.3	1.5	12.8	2.8	0.8	6.8
PEClocal - air[3]	mg/m^3	**7.6E-04**	**2.9E-04**	**2.9E-04**	**9.9E-04**	**3.5E-04**	**1.7E-03**	**6.5E-04**	**1.9E-04**	**1.3E-03**

1) From Table 3.5
2) In calculating PEC_{local} the $PEC_{regional}$ for surface water = 2.2 µg/l is added as a background level (see Table 3.56).
3) In calculating PEC_{local} the $PEC_{regional}$ for air =7.5 ng/m^3 is added as a background level (see Table 3.56).
4) Values with ">" are based on a calculated surface water concentration that is above the water solubility. This probably underestimates the concentration in the sediment.
5) The effluent discharge rate for STP was set to default (2*10^6 l/d).

Table 3.52 Site specific local PECs for polymer processing/formulation

Life cycle stage	Site	Air[1] (µg/m³)	Surface water[2] (µg/l)		Sediment (mg/kg dwt)	Agricultural soil (mg/kg dwt)	Oral worm (mg/kg wwt)
			during emission	annual average			
2a calendering	F3	0.16	2.3	2.3	21	0.06	0.05
	S4	0.58	2.2	2.2	218	0.06	0.05
	S6	0.35	2.4	2.3	22	0.2	0.1
2b extrusion compounding	S5	0.019	2.2	2.2	21	0.02	0.04
2c extrusion product	F2	0.16	2.5	2.4	23	0.3	0.14
	F7	0.08	2.3	2.3	21.5	0.1	0.07
	I9	0.03	2.4	2.4	22.5	0.2	0.11
	S11	0.02	2.2	2.2	21	0.02	0.04
2d plastisol spread coating	F1	0.24	2.2	2.2	21	0.05	0.05
	S8	0.97	2.2	2.2	21	0.09	0.06
2? not known	Es12	0.20	2.2	2.2	21	0.02	0.04
	Es13	0.06	2.2	2.2	21	0.04	0.05

1) In calculating PEC_{local} the PECregional for air =7.5 ng/m³ is added as a background level (see Table 3.56).
2) In calculating PEC_{local} the PECregional for surface water = 2.2 µg/l is added as a background level (see Table 3.56).

Table 3.53 PEC in aquatic biota (secondary poisoning). Life cycle stage 2: Polymer Formulation/Processing

	Polymer processing sites		**PECwater[1] annual average (ug/l)**	**PEC oral- aquatic[2] (mg/kg wwt)**		
				Fish[3]	**Mussel[3]**	**invertebrates[4]**
2a	Calendering	generic	9.7	2.2	15	8
		site F3	2.3	1.9	5.7	6.2
		site S4	2.2	1.9	5.5	5.9
		site S6	2.4	1.9	6	6.5
2b	Extrusion-compound	generic	5	2.2	9	8
		site S5	2.2	1.9	5.5	5.9
2c	Extrusion-products	generic	5	2.2	9	8
		site F2	2.4	2.0	6.2	6.8
		site F7	2.3	1.9	5.7	6.2
		siteI9	2.4	1.9	6	6.5
		site S11	2.2	1.9	5.5	5.9
2d	Spread coating	*average air cleaning – generic*	12	2.2	18	8
		with air cleaning – generic	5.6	2.2	102	8
		without air cleaning – generic	30.8	2.2	41	8
		site F1	2.2	1.9	5.5	5.9
		site S8	2.2	1.9	5.5	5.9
2e	Other plastisols	*average air cleaning – generic*	8.5	2.2	13	8
	Car undercoating	*with air cleaning – generic*	4	2.2	83	8
		without air cleaning – generic	17.5	2.2	25	8
2?	Not known	site Es12	2.2	1.9	5.5	5.9
		site Es13	2.2	1.9	5.5	5.9

1) Annual average. From Table 3.51 and Table 3.52
2) BCF: fish=840; mussel=2500; zooplankton=2700. The water solubility 3 µg/l is used as a limit for the calculation of PECoral aquatic fish and PECoral aquatic zooplankton, i.e. when PECsurface water exceeds the water solubility the water solubility is used for calculating PECoral aquatic. For PECoral aquatic mussels however, the calculated PECsurface water is used.
3) PECs based on the assumption that 50% of prey is sourced from the local environment and 50% from the regional environment, according to TGD
4) Predator fish are assumed to be stationary and consume all prey locally. Thus, $PEC_{oral\ invertebrates}$ is calculated based only on PEClocal surface water.

Table 3.54 Local PECs for life cycle stages 3-8, generic values except for site F10

Life cycle stage	Product type	Handling type	Air[1] (mg/m³)	STP (mg/l)	Surface Water[2] during emission (ug/l)	Surface Water[2] annual average (ug/l)	Sediment[3] (mg/kg dwt)	Agricultural soil soil (mg/kg dwt)	Agricultural soil pore water / ground water (ug/l)	Natural Soil soil (mg/kg dwt)	Natural Soil pore water (ug/l)	Urban/ Ind. soil (mg/kg dwt)
LOCAL												
3a	Sealants, adhesives	Formulation	**2.1E-03**	**1.26**	**104**	**86**	> 966	**103**	**26**	**35**	**11**	b
		site F10	**0.29E-03**	**b**	**2.2**	**2.2**	**21**	**0.07**[5]	**8E-03**	**0.04**	**0.01**	b
3b	…	Processing/Application	**4.9E-05**	**0**	**2.2**	**2.2**	**21**	**0.07**[5]	**5.1E-3**	**0.018**	**5.6E-3**	b
4a	Lacquers and paints	Formulation	**2.8E-04**	**0.16**	**15**	**13**	**> 144**	**13**	**3.5**	**4.6**	**1.4**	b
4b	*water based*[4]	Processing/Application	**7.7E-06**	**0.01**	**3.2**	**3.0**	**> 30**	**1.0**	**0.26**	**0.35**	**0.1**	b
5a	Printing inks	Formulation	**3.35E-04**	**0.19**	**16**	**13.3**	**> 149**	**15.5**	**4**	**5.3**	**1.6**	b
5b		Processing/Application	b	b	b	b	b	b	b	b	b	b
6a	Ceramics	Formulation	**1.3E-05**	**0.007**	**2.8**	**2.7**	**26**	**0.55**	**0.14**	**0.20**	**0.06**	b
7	Polymer + Non-polymer	Municipal STP	b	**0.03**	**4.8**	**4.8**	**> 44**	**2.6**	**0.67**	**0.89**	**0.27**	b
8a	Waste	Paper recycling	**8.0E-06**	**0.04**	**5.2**	**4.2**	**> 48**	**3.0**	**0.77**	**1.0**	**0.3**	b
8b	Waste	Car shredder	**2.3E-05**	**0**	**2.2**	**2.2**	**21**	**0.07**[5]	**0.005**	**0.016**	**0.005**	b
8c	Waste	Incineration	**9.1E-05**	**0**	**2.2**	**2.2**	**21**	**0.07**[5]	**0.006**	**0.022**	**0.007**	b

1) In calculating PEC_{local} the PECregional for air =7.5 ng/m³ is added as a background level (see Table 3.56).
2) In calculating PEC_{local} the PECregional for surface water = 2.2 µg/l is added as a background level (see Table 3.56).
3) Values with ">" are based on a calculated surface water concentration above the water solubility. This probably underestimates the concentration in the sediment.
4) Water based systems have 5 times higher releases to waste water than solvent based and is therefore selected as a local worst case scenario. Water related PECs as calculated by EUSES are therefore increased 5 times.
5) For these scenarios the EUSES model calculates a PEC_{local} that is lower than $PEC_{regional}$. That figure is therefore replaced with $PEC_{regional}$.
b) Not applicable

Table 3.55 Local PEC in earthworm and aquatic biota (secondary poisoning). Life cycle stage 3-8.

Life cycle stage generic values except for site F10		PEC Oral worm (mg/kg wwt)	PECwater[1] (µg/l)	PEC oral-Aquatic[2] (mg/kg wwt)		
				Fish[3]	Mussel[3]	invertebrates[4]
3a	Sealants, adhesives – formulation	38.6	86	2.2	133	8
	adhesives site F10	0.04	2.2	1.9	5.5	5.9
3b	Sealants, adhesives - processing	0.037	2.2	1.8	5.5	6
4a	Lacquers and paints - formulation	5.1	13	2.2	21.5	8
4b	Lacquers and paints – processing[5]	0.4	3.0	2.2	6.80	8
5a	Printing inks - formulation	5.8	13.3	2.2	18	8
6a	Ceramics - formulation	0.24	2.7	2.1	6.2	7.6
7	Municipal STP	1.0	4.8	2.2	8.8	8
8a	Paper recycling	1.15	4.2	2.2	9.3	8
8ba	car shredder	0.036	2.2	1.8	5.5	6
8cb	incineration station	0.037	2.2	1.8	5.5	6

1) Annual average. From Table 3.54
2) BCF: fish=840; mussel=2,500; zooplankton = 2,700. The water solubility 3 µg/l is used as a limit for the calculation of PECoral aquatic fish and PECoral aquatic zooplankton, i.e. when PECsurface water exceeds the water solubility the water solubility is used for calculating PECoral aquatic. For PECoral aquatic mussels however, the calculated PECsurface water is used.
3) PECs based on the assumption that 50% of prey is sourced from the local environment and 50% from the regional environment, according to TGD
4) Predator fish are assumed to be stationary and consume all prey locally. Thus, $PEC_{oral\ invertebrates}$ is calculated based only on PEClocal surface water.
5) Water based systems have 5 times higher releases to waste water and is therefore selected as a local worst case scenario. Water related PECs are then increased 5 times.

3.1.3.2 Continental and Regional PECs

The regional and continental PECs were calculated with the EUSES model. The results are presented in **Table 3.56**.

Table 3.56 Calculated regional and continental PECs

Scenario	Air (mg/m³)	Surface water (µg/l)	Sediment (mg/kg dwt)	Agricultural soil (mg/kg dwt)	Soil pore water/ ground water (µg/l)	Natural soil (mg/kg dwt)	Soil pore water (µg/l)	Urban/Ind. Soil (mg/kg dwt)
Regional	7.5E-06	2.2	33.7	0.07	0.02	0.015	-	3.2
Continental	1.6E-06	0.3	4	0.006	0.002	0.003	-	0.33

3.1.4 Monitoring data

A large number of monitoring studies on DEHP are available. In studies earlier than the eighties, problems concerning contamination of samples via the sampling and analysis equipment may not

have been fully considered in the design of the investigations, and the results have to be interpreted with caution.

3.1.4.1 Measured levels in the aquatic compartment

Surface water and sediment

The monitoring data in surface water and sediment are summarised in tables 10 - 12, Annex 1. Reports from earlier than 1985 are excluded from the data summary on surface water and sediment. Several studies are also excluded due to lack of information on pollution status or/and on sampling or analytical methods. Principally, only studies where the original reports were available are included in the surface water and sediment section. A majority of the data is assumed to represent regional or continental concentrations. However, it cannot be excluded that some of the measured DEHP concentrations may result from an unknown local point source.

A monitoring project on surface water and sediment was carried out in Sweden during late winter/spring in 1996 (Parkman and Remberger, 1996). Water- and sediment samples were taken from two unpolluted lakes (Härsvatten and Fräcksjön) and from waters affected by diffuse discharges from five cities in Sweden (Stockholm, Karlstad, Uppsala, Norrköping and Örebro). Some of the sampling sites were situated downstream sewage plants. A similar project for monitoring of DEHP in sediments was performed in 1994 (Parkman and Remberger, 1995). Seven sampling sites were situated in unpolluted lakes, ten sites downstream Gullspångsälven – Vänern – Göta älv with increasing anthropogenic impact. One site was situated in Riddarfjärden, close to a dumping site for snow masses from the centre of Stockholm. Finally, samples were taken upstream and downstream of two point sources – processing sites - in connection with Svartån and Ronnebyån in the south of Sweden. However, little information is available about the discharge rates from the two point sources.

Water samples were taken at a depth of 1 m below the surface, using a pre-heated glass bottle attached to an iron rod. The water was not filtered prior to analysis. Sediment samples were taken by means of a Benell-corer, modified for phthalate sampling by exchanging the standard plastic and rubber parts with parts made of Teflon. Surface sediment from the upper two centimetres was analysed, as well as a deeper layer. In one lake (Härsvatten), a depth profile of a sediment core was analysed.

Analysis of whole (not filtered) water samples was performed after solid phase extraction by means of GC. Sediment samples were extracted with acetonitrile, hexane/acetonitrile and analysed by GC. Surface water concentrations in unpolluted lakes ranged from 0 – 0.013 µg/l (mean 0.009 µg/l, n=6). Levels of DEHP in surface waters from urban areas ranged between 0.010 and 0.33 µg/l.

In the unpolluted lakes, surface sediment concentrations were 0.07 – 0.60 mg/kg dwt. The levels were generally lower in deeper layers than in the surface sediment. In the depth profile of lake Härsvatten, levels of DEHP decrease significantly with depth – from 0.6 mg/kg dwt in the upper centimetre to between 0.05 and 0.08 mg/kg dwt below 5 cm depth. The uppermost 2.5 cm of the sediment was claimed to represent the last 25 years of deposition. In the Gullspångsälven and Göta Älv river systems, DEHP levels clearly increased downstream when expressed per organic content of the sediment.

In urban areas affected by diffuse discharge of DEHP, sediment concentrations in the upper 2 cm layer ranged between 0.65 and 1.22 mg/kg dwt. In sediment from Riddarfjärden, close to the

dumping site for snow masses, the concentration of DEHP was 2.5 mg/kg dwt. Downstream the point sources in Svartån and Ronnebyån, levels of 47 and 33 mg/kg dwt were measured in the upper layers. In an earlier study (Thurén, 1986, samples taken in 1983) maximum sediment concentrations of 1480 and 628 mg/kg dwt, respectively, were measured at the same sites. The differences can be explained by the decreased use levels of DEHP and the introduction of wastewater treatment on the two processing sites, respectively.

A similar monitoring project was carried out simultaneously in freshwater, seawater, sediment and domestic sewage in Norway (Braaten et al, 1996). Water and sediment samples were taken on possible points of discharges from PVC using industry (11 localities in the Oslofjord, Drammensfjord, Grenlandsfjord and the Idefjord) and from unpolluted reference areas (lakes: Mjösa, Femunden, Lundevatn and Heddalsvatn, marine sites: Langesundsbukta, and Faerder).

Single water samples were taken at a depth of 1 m below the surface, using a pre-heated glass bottle attached to a steel rod. Sediment samples were taken in triplicate by means of a Limnos sediment sampler, consisting of a tube made of polycarbonate. Surface sediment from the upper two centimetres and from 18 - 20 cm depth were analysed. The analyses were performed similarly as described in Parkman and Remberger, 1996.

In seawater the levels of DEHP ranged between < 0.018 and 0.38 µg/l. Concentrations in lake water samples from unpolluted areas were < 0.060 - 0.18 µg/l.

The highest concentrations of DEHP in marine sediments were found outside a STP of Oslo, 6.6 mg/kg dwt. In Langesundsbukta and Faerder (marine reference stations), 0.034 and 0.08 mg/kg dwt were measured. The upper layer of lake sediments from unpolluted areas contained 0.042 - 0.80 mg/kg dwt.

In a recent study, carried out in Denmark during 1996-98 (Boutrup et al, 1998), levels of a large number of chemical substances in different environmental compartments were investigated. Samples of surface water and sediment were taken in three rivers, four lakes and on five marine locations in the county of Aarhus, and in marine locations in the South Western part of the inner Danish waters.

Water samples were taken by means of pre-heated glass bottles. Sediment samples from lakes were collected using a plexiglass/aluminium tube, while marine samples were taken by a Hapsbund-sampler. The upper 2 cm was transferred to glass bottles with a steel spoon. On one marine site, sediment from 19 - 21 cm depth was also analysed, representing precipitated sediment from ca 1900.

All samples were analysed with GC/MS-SIM after extraction in dichloromethane. The concentrations found in water were < 0.2 - 0.87 µg/l, with the highest levels observed during increased water flow. In river sediment, the concentrations were 0.075 - 6.9 mg/kg dwt, in lake sediment 0.31 - 2.5 mg/kg dwt. Marine surface sediment in the county of Aarhus contained < 0.05 - 5.4 mg/kg dwt, the highest value found in the port of Aarhus. In the sediment layer of 19-21 cm depth, no DEHP was detected (limit of detection 0.05 mg/kg dwt). In sediment samples from in the South Western part of the inner Danish waters, the measured levels ranged between 0.031 and 16 mg/kg dwt (limit of detection 0.01 mg/kg dwt), with the highest level observed in the Vejle fjord and the lowest concentrations in the open sea areas.

In a monitoring study performed in the Netherlands in 1992-93 (Bodar, 1996), sediment samples were taken from five different sites in major rivers and ten different sites in small rivers. Besides, samples from 11 sites close to producers/processors of phthalates and from five sites alongside

highways were analysed. Water samples were taken once per month during three years from the rivers Rhine and Meuse. Samples were also taken from eight wastewater treatment plants.

In the major rivers, DEHP concentrations in sediment between 1 and 4 mg/kg dwt were measured. In the small rivers, the levels were close to the limit of determination (0.5 mg/kg dwt). On sampling locations close to producers/processors, the concentrations of DEHP were < 0.5-4.5 mg/kg dwt, and alongside highways < 0.5 - 25 mg/kg dwt. Water from Rhine and Meuse contained < 0.1 - 1.0 µg/l and < 0.1 - 4.2 µg/l, respectively. No details on discharge levels, sampling or analytical methods were available in the summary report.

A monitoring study on DEHP was performed in Northrhein Westfalen an industrial region in Germany during a two-year period (Furtmann, 1993). Samples of water were taken from eleven locations every four weeks over the test period. The sample sites were situated in the rivers Rhine, Lippe, Ruhr, Emscher, Erft, Sieg and Wupper. In addition, water and sediment samples were taken from the river Weser and its tributaries, from canals in Northrhein-Westfalen (which were anticipated to be of low industrial influence) and in ports of the Rhine. In the Rhine and its tributaries, phthalate levels adsorbed by suspended matter were also calculated. On two locations in rivers Rhine and Lippe, depth profiles of sediment were dated and analysed for DEHP. The stratified sample from river Lippe was taken near a known phthalate producing industry. Water samples were taken by pre-heated glass bottles, deactivated by lining with 2, 2, 4-trimethyl pentane. Sediment samples were - in most cases - taken using a dredging grab from a ship. On these locations, no distinction between recent and older sediment could be made, since the depth profile was disturbed. Where stratified sediment was desired, samples were taken by means of sediment boxes, sunk into the river. Only stainless steel tools were used. Analysis of whole water samples was performed using solid phase extraction and GC-mass selective detector (SIM). Sediment samples were dried, extracted with ethyl acetate and analysed by means of GC.

The levels of DEHP in surface waters ranged between 0.1 and 10 µg/l. The mean concentration of 216 individual samples from Rhine and its tributaries was 0.85 µg/l and the 90^{th} percentile was 1.6 µg/l. Two sampling sites (river Emscher and Bad Honnef in Rhein) had markedly higher mean concentrations than the other 9 sampling points probably due to local emissions. The DEHP concentration in Weser and its tributaries average ranged from 0.1 to 4 µg/l with an average concentration of 0.94 µg/l (n=27). The concentrations in the canals ranged from 0.07 to 0.48 µg/l with a mean of 0.22 µg/l (n= 12).

The contents of DEHP in suspended material in Rhine and its tributaries were 0-282 mg/kg dwt. Sediment levels of DEHP in river Weser and its major tributaries were 0.1 - 8.9 mg/kg dwt, in Rhine 1.8 - 18 mg/kg dwt. In canals in Nordrhein-Westfalen, the levels were 0.2-3.4 mg/kg dwt, and in industrial ports 0.35 - 21 mg/kg dwt. In the stratified samples from Rhine and Lippe, the first occurrence of DEHP was observed in sediment dated to 1945-52 and 1935-37, respectively. Peaks were observed in the seventies – in Rhine the highest concentration was 3.5 mg/kg dwt, in Lippe (near phthalate producer), the maximum concentration was 24 mg/kg dwt. The decreased concentrations in more recent sediment layers are probably due to improved efficiency of the wastewater treatment.

In a follow up study performed 1998 – 2000 (Alberti et. al. 2001) the rivers in Northrhein Westfalen were investigated again (Rhein, Lippe, Emscher, Erft, Ruhr, Sieg, Wupper and Niers). The river Wupper was given special attention and samples were taken at 24 sites along the river. In addition to this surface water samples were taken from several major German rivers. Water and sediment samples from two rainwater basins connected to motorways, suspended matter from Rhine and Wupper, and sediment samples from three ports in Rhine were also analysed for phthalates.

The DEHP concentrations in river water in this study were similar to those measured in 1993. The concentrations ranged from non-detectable to 3.1 µg/l with an overall mean of 0.63 µg/l and a 90th percentile of 1.48 µg/l (N= 77). The concentrations in the Northrhein Westfalen rivers (excluding Wupper) ranged from non-detectable to 2µg/l with a mean of 0.57 µg/l and a 90th percentile of 1.56 µg/l (N=22). As in the first study some of the sampling points e.g. river Emscher had markedly higher mean concentrations than the other sampling points. The mean concentration for the other German rivers was 0.48 µg/l and the 90th percentile was 0.98µg/l (N=27). The DEHP concentrations along river Wupper ranged from 0.12 to 3.1 µg/l with a mean of 0.82µg/l and a 90th percentile of 1.67µg/l (N=26). The DEHP concentration in motorway run-off was extremely high, 240µg/l at one site and 560 µg/l at the other. Also the concentrations in sediment were extreme at the two motorway sites 1,300 and 660 mg/kg dwt, respectively. The concentration in suspended matter at the two sampling sites in Rhine was 1.6 and 1.9 mg/kg dwt, respectively and 9.5 mg/kg dwt at the sampling site in Wupper. The concentration in sediment from the three harbours in Rhine was 3.7, 14 and 50 mg/kg dwt, respectively.

In a monitoring study on micro-organic compounds in water and sediments carried out in the UK (Long et al, 1998), samples were collected from six fresh water tributaries of the Humber river (Trent, Aire, Calder, Don, situated in urban and industrial areas; Ouse and Swale, situated in agricultural areas). Samples were taken on four occasions at quarterly intervals from July 1995-1996.

The method for sampling of water was not described. Suspended- and "bed" sediment were collected using a field-based continuous flow centrifugation system. Samples of suspended sediment were collected from a total volume of 360 litres of river water. Bed sediment was collected by "hoovering" the top few centimetres of the riverbed from an area of approximately 5 m^2. Water samples were extracted with dichloromethane, subsequently dried and reconstituted in ethyl acetate. Sediment samples were supercritical-fluid extracted prior to analysis with GC/MS.

DEHP was shown to be present in all water- and sediment samples analysed. The whole water concentrations ranged between 0.36 and 21 µg/l (mean 6.6 µg/l) in industrial and urban areas, and between 0.74 and 21 µg/l (mean 5.3 µg/l) in agricultural areas. The concentrations were significantly higher in samples taken in February and May compared with those from July and October, probably as a result of the higher flow, which enhance the levels of suspended matter in the water. The levels of DEHP in "bed" sediment were between 3.4 and 19.4 mg/kg dwt (mean 7.8 mg/kg dwt) in industrial and urban areas, and between 0.23 and 17.9 mg/kg dwt (mean 6.1 mg/kg dwt) in agricultural areas. In suspended sediment, the corresponding values were 3.8-115 mg/kg dwt (mean 34.5 mg/kg dwt).

Desideri et al, 1991 determined the levels of a number of organic compounds in Antarctic sediment. Samples were taken on 14 sites at depth between 20 and 1,100 metres, by means of a bucket or with a dredger. Samples taken near the coast were taken by an under-water diver. Analysis was performed with GC after extraction with *n*-hexane/methylene chloride, methanol and water. DEHP was shown to be present in all sediment samples, at concentrations ranging from 0.007 - 0.14 mg/kg.

Measurements of the concentrations of DEHP and other phthalates in Dutch sediment samples were performed by ALcontrol (1999). Single or duplicate sediment samples were taken from 25 sites within the Netherlands. In addition samples were taken in the vicinity of two production sites A and B located outside the Netherlands. The samples were extracted with dichloromethane and the analyses were carried out on an LC-LVI-GCMS. Measures were taken to prevent contamination of the samples prior to analysis. The detection limit was 0.025 mg/kg dwt.

The levels of DEHP in samples that could not be attributed to local emission sources ranged between not detectable (< 0.025 mg/kg dwt) and 1.7 mg/kg dwt (mean of duplicate samples). The mean was 0.29 mg/kg dwt and the 90th percentile was 0.62 mg/kg dwt. The highest regional concentrations were observed in the major Dutch river Meuse (Maas). In the sediment samples taken close to production site A the concentration was 2 and 7.6 mg/kg dwt at two different sampling points, whereas the concentration in the sediment of an oxidation pond which is a part of the WWTP system was ca 8 mg/kg dwt. It can be noted that the concentration of DIDP in the sediments was far higher (approximately 4-5 times) so it may be questioned if this site is producing DEHP. The concentration in sediments at site B was 0.46 mg/kg dwt 2 km upstream and 0.25 mg/kg dwt 2 km downstream the plant.

In November 2000 sampling of sediments was performed by RIVM at 5 different locations in the Netherlands (RIC 2001a). Two of the sites had been sampled before in the study by Alcontrol 1999. The samples were analysed by RIC using ultrasonic extraction with cyclohexane and direct GC-MS analysis. The concentration in the sediments was: 3.3, 1.3 (0.2 in Alcontrol 1999), 1.1 (0.7 in Alcontrol 1999), 2.6 and 1.9 µg/kg dwt.

In Basseres et al (2000), surface water and sediment samples were taken near a production site in a small French river. In water, concentrations between 1.1 and 7.4 µg/l was detected downstream the production site. In sediment the concentrations were 40 – 146 mg/kg dwt. However, similar levels of DEHP were also observed in water and sediment upstream this point source since other DEHP using industries are situated nearby. Therefore, the data are difficult to interpret.

In a survey in the river Viskan in southwest Sweden sediment samples were taken at 13 sites along the river from its origin to the river mouth (Golder Grundteknik 2000). The sediments were analysed for metals and a large number of organic substances including phthalates. DEHP was found in all samples. The concentrations ranged from < 0,1 mg/kg dwt to 100 mg/kg dwt. The highest concentrations were found at two sampling sites downstream the city of Borås. These sites have a high level of historical pollution mainly due to discharges from textile industries and also receive discharges from a big municipal STP. At the site closest to Borås where also a depth profile was taken the DEHP concentration in the 10-20 cm layer was 100 mg/kg dwt whereas in the upper 10 centimetres it was 40 mg/kg dwt, indicating that the releases have decreased. In the 20-30 cm layer the DEHP concentration was 57 mg/kg dwt and in the 30-40 cm layer 27mg/kg dwt.

Groundwater

Available monitoring studies on DEHP in groundwater are summarised in Table 15, Annex 1. In groundwater associated to non specified contaminated land (Hutchins et. al. 1985; Anzion, 1987), measured concentrations of DEHP ranged between 0.05 and 45 µg/l. In one study (Henriksson 1997), agricultural soil was treated with STP sludge prior to groundwater sampling. DEHP levels in groundwater were between 0 and 510 µg/l. In groundwater close to a landfill in Sweden (Oman and Hynning 1993), the concentration of DEHP was 1797 µg/l. However, due to the sampling technique, errors might have occurred.

In a monitoring study performed on DEHP in private and public water supply boreholes in UK (Kenrick et. al. 1985), DEHP was found in 7 out of 11 samples. The average measured concentration was 0.07 µg/l.

3.1.4.1.1 Calculated PECs compared with monitoring data Aquatic compartment

Surface water

The overall mean of detected concentrations in river waters can be calculated to 1.3 µg/l. In lake water, the concentrations were lower, with a mean of 0.08 µg/l. The difference is probably due to the higher levels of suspended matter in flowing waters. In marine surface waters in Norway the concentration of DEHP was below 0.1 µg/l except for one sampling station close to a municipal STP where the concentration was approximately 0.4µg/l.

Measured concentrations of DEHP in surface waters affected by diffuse pollution from industry and urban areas range from non-detectable to 21 µg/l. In the most extensive study (Furtmann, 1993) the concentrations in river Rhein and some of its tributaries in Nordhrein Westfalen ranged from 0.1 – 10 µg/l. The mean concentration of 216 individual samples was 0.85 µg/l and the 90th percentile was 1.6 µg/l. The further measurements performed in Northrhein Westfalen in 1999 – 2000 (Alberti et. al. 2001) indicated slightly lower values. The concentrations ranged from non-detectable to 2µg/l with a mean of 0.57 µg/l and a 90th percentile of 1.56 µg/l (N=22). The number of samples was much lower and did not, as in the first study, take into account the seasonal variation so the conclusion is that the DEHP concentration in surface waters in Northrhein Westfalen has not changed since 1993. The overall mean for all measurements made in Northrhein Westfalen rivers 1993 – 2000 is 0.83 µg/l. Since Northrhein Westfalen is a highly industrialised and densely populated area this figure is judged to be representative for a worst case regional situation. The EUSES model seems to slightly overestimate the surface water concentration and calculates a regional concentration of 2.2µg/l. Based on the large amount of data available it is concluded that the measured mean value from Northrhein Westfalen rivers of 0.8 µg/l will be used in the risk characterisation in addition to the $PEC_{regional}$ calculated by EUSES.

In one study (Basseres et al, 2000) samples were taken near a production site, but since other phthalate using industries were situated nearby the measured concentrations of DEHP (1.1-7.8 µg/l) cannot be attributed to this single point source only. Calculated local concentrations for the production sites are mostly based on site specific data and range from 2.2 to 220 µg/l. No measured data are representative of processing and formulation sites. Calculated local concentrations range from 4.4 – 37 µg/l.

In remote lakes in Sweden and Norway, which were expected not to be affected by industry or other human activities, DEHP concentrations around 0.01 µg/l were measured. This level can be compared with the calculated continental PEC of 0.3 µg/l**.**

Sediment

Measured concentrations of DEHP in surface sediment from rivers and lakes situated in industrial or urban areas where no DEHP using industries were specified ranged between 0.04 and 21 mg/kg dwt. However, judging from all available data it seems plausible that the sites with the highest concentrations are affected by point sources or historically have been affected by point sources. The mean of 58 sites with freshwater sediment was 5.2 mg/kg dwt, to be compared with the calculated regional PEC of 33.7mg/kg dwt. In marine sediments associated to industrial and/or urban areas, measured concentrations averaged 1.5 mg/kg dwt.

In surface sediment originating from canals in Germany where a limited discharge of DEHP was anticipated, the concentrations ranged between 0.15 and 3.4 mg/kg dwt (mean 1.5 mg/kg dwt, n=10).

Several of the sediment sampling sites were situated in the neighbourhood of DEHP using industries. The concentrations ranged from approximately 0.3 mg/kg dwt to 1,480 mg/kg dwt. The two highest concentrations (668 and 1,480mg/kg dwt) were measured close to a Swedish point source in 1983. The concentrations at these sites had declined to 33 and 47 mg/kg dwt, respectively in 1994. Outside a DEHP producer in France concentrations of 40 – 146 mg/kg sediment dwt were measured. These concentrations are probably not solely attributed to this point source, as also other phthalate using industries are present in the area. At a site in the river Viskan downstream the city of Borås in Sweden high concentrations of DEHP were measured. In the upper 10 centimetres of the sediment the concentration was 40 mg/kg dwt and in the 10-20 cm layer the concentration was 100 mg/kg dwt. This site has a high historical pollution due to direct discharges from textile industry and is now a recipient for a municipal STP. In sediment from three harbours in Rhine the DEHP concentrations ranged from 4 – 50 mg/kg dwt. In marine sediments close to DEHP processing industries in Norway the concentrations ranged from 0.1 to 3.2 mg/kg dwt. The calculated concentrations for the different production sites ranges from 21 to 2,060 mg/kg dwt the majority based on site specific emission data. The calculated local sediment concentrations for polymer and non-polymer processing sites are based on default emission factors and range from 41 to 345 mg/kg dwt.

DEHP was also found in sediment from areas far away from industry or dense human populations. In samples of Antarctic sediment levels from 0.007- 0.14 mg/kg (mean 0.027 mg/kg (dwt?), n=14) were observed, indicating a significant long-range transport of DEHP. In freshwater sediment from more remote areas in Sweden, Norway and Denmark, the concentrations ranged between 0.008 and 0.8 mg/kg dwt (mean 0.25 mg/kg dwt, n=18). These data can be compared with the calculated continental PEC in sediment, 4mg/kg dwt**.**

In general, measured concentrations of DEHP were lower than the corresponding calculated levels**.** A value of 5 mg/kg dwt will be used to override the EUSES calculated $PEC_{regional}$ of 33.7 mg/kg.

3.1.4.2 STP

There are many data available on DEHP concentrations in wastewater and sewage sludge (see Table 16 and 17 in Annex 1). Most studies concern municipal wastewater treatment plants but there are also a few data concerning industrial effluents. No information on DEHP concentrations in influent water to industrial STPs or DEHP concentrations in industrial sewage sludge is available

Municipal wastewater

Flow proportional 24 h samples were taken on the effluent wastewater from 5 different housing areas with low industrial load. Furthermore samples were taken from the two main inlet tubes to (> 95% of the inflow) and the outlet from Henriksdal sewage treatment plant in Stockholm, Sweden in February and March 1989 (Stockholm Vatten, 1990). The DEHP concentration in effluent water from the housing areas was 3 – 15 µg/l. In the two inlet tubes the DEHP concentrations were 6 – 11 µg/l on weekdays and 4 – 6µg/l on a weekend. The DEHP concentration in the outlet was below the detection limit of 1µg/l. Consequently the removal rate was > 90%.

In a follow up study (Stockholm Vatten, 1991a) samples were taken from two of the housing areas and from the two inlet tubes and the outlet in February 1991 (weekday 13/2 –14/2, weekend 17/2 – 18/2). The concentrations of DEHP was higher this time: 26 - 49µg/l in the

housing areas, 39 and 47 µg/l in the two inlets, respectively on the weekday and 34 and 46 µg/l, respectively on the weekend. The concentration in the outlet was 28 µg/l (weekday) and 15 µg/l (weekend). The removal rates were approximately 40% on the weekday and 67% on the weekend. The reason for the difference in DEHP concentrations between the two studies is not known. It shall be noted however, that different laboratories performed the analyses in 1989 and 1991.

In a third study samples from three inlet tubes to and the outlet from Bromma sewage treatment plant in Stockholm were analysed for DEHP (Stockholm Vatten, 1991b). The samples were collected in November – December 1990. The mean DEHP concentration in the inlet tubes was 32 µg/l whereas it was 1.8 µg/l in the outlet indicating a removal rate of approximately 95%.

The influent and effluent wastewater from three Danish sewage treatment plants (Skœvinge, Avedøre and Marselisborg) was analysed for its content of metals and organic substances including DEHP (Grüttner and Jacobsen, 1994). The samples were taken in October 1992. The DEHP concentrations in the influent water was 247, 122 and 223 µg/l in Skœvinge, Avedøre and Marselisborg, respectively, whereas the effluent water contained 5.2, 23 and 0.5 µg/l resulting in removal rates of 98, 81 and 99%. In July 1995 a follow up study was performed on the same three STPs (Grüttner et al., 1995). In this study two other laboratories performed the analyses (the samples from Marselisborg were also analysed by the laboratory responsible for the 1992 analyses). Compared to 1992 the DEHP concentrations in influent water were lower while the effluent concentrations were on the same level. There was little difference between the analysis results from the different laboratories. The DEHP concentrations in the influent water to Skœvinge was 33 and 14µg/l, to Avedøre 49 and 35 µg/l and to Marselisborg 39, 26 and 28 µg/l, respectively. The DEHP concentrations in the effluent water from Skœvinge was 4 and 4µg/l, from Avedøre 28 and 10 µg/l and from Marselisborg 0.5, < 2.5 and < 7 µg/l, respectively indicating removal rates in the range of 70 – 90% at Skœvinge, 40 – 70% at Avedøre and > 75-99% at Marselisborg.

In a recent Danish study from the Århus area (Boutrup et al., 1998) samples were taken from the influent and effluent water from 2 different STPs (Søholt and Viby) in 1996. The mean DEHP concentration (n=3) in the influent was 33.3 µg/l at Søholt and 35 µg/l at Viby. In the effluent from the two STPs the mean concentrations were 2.4 and 1 µg/l, respectively, indicating removal rates of 93 and 97%.

In a Norwegian study (Braaten, 1996), the DEHP concentrations in influent wastewater to three STPs: Bekkelaget, Fugelvik and Slemmestad (VEAS) was 6.3, 12.8 and 15 µg/l, respectively. The effluent contained 0.08, 0.13 and 0.07 µg/l respectively, indicating removal rates around 99% for all three STPs.

Furtmann (1996), reports an influent concentration of 25 µg DEHP/l in June 1992 from a sewage treatment plant dominated by household wastewater. The effluent concentration of DEHP was 0.54 µg/l; consequently the removal rate was 98%. In a STP dominated by industrial wastewater the influent concentration of DEHP was 71µg/l in June 1992. The effluent contained 0.9 µg/l giving a removal rate of 99%.

Alberti et al. (2001), reports effluent concentrations ranging from 0.05 to 1.0 µg/l from 8 different municipal STPs. The mean concentration was 0.38 µg/l.

UK water industry research limited has made a study as part of a research programme to improve the water industry's understanding of the presence and origins of priority substances in sewage and to prepare for the Water Framework Directive.

The urban catchment study involved sampling and analysis of foul water samples from separate subcatchments of the sewerage system representative of old and new housing, of the town centre and of light industrial estate. Runoff samples were taken from a housing estate and the light industrial estate.

DEHP was detected in all samples, including runoff. Concentrations in the new housing estate wastewater were five times higher (average of 57 µg DEHP/l) than that of the older estate (average of 9 µg DEHP/l) and the light industrial estate (average of 6 µg DEHP/l), suggesting that leaching from newly installed plastics is a significant diffuse input to wastewater treatment works.

Industrial wastewater

There are also some studies available where purely industrial wastewater has been analysed.

Källquist et al. (1991) detected 56.9 µg DEHP/l in the effluent wastewater from the Swedish DEHP producer Neste Oxo in June 1990. In a later study flow proportional 24-hour samples of effluent wastewater from Neste Oxo was collected during the period 15 November to 21 November 1997 (Solyom and Ekengren, 1997). The concentration of DEHP was 0.08 µg/l.

In November and December 1990 Stockholm Vatten took samples from three tubes leading wastewater from two industrial areas (no households in the area) to the municipal STP in Bromma, Sweden (Stockholm Vatten, 1991b). The concentrations of DEHP in the wastewater from the three tubes were 1,800, 28 and 55 µg/l, respectively. The authors have no explanation to the very high concentration in one of the tubes.

Alberti et al. (2001) analysed effluent wastewater from different industrial STPs and also leachates from landfills in Germany. The DEHP concentrations in industrial effluents ranged from 0.1 – 340 µg/l. The highest concentrations 17 and 340 µg/l were measured in effluent wastewater from two leather-producing sites. With these two sites excluded the range was 0.1-3.1 µg/l (mean 0.97 µg/l; N= 17). At two sites also influent wastewater classified as "mixed waste water from chemical industry" was analysed. The concentrations were 45 µg/l and 17 µg/l in influent wastewater. The corresponding concentrations in the effluent were 0,08 and 0,9 µg/l indicating removal rates of > 99% and 95%, respectively. The concentrations in landfills leachates varied from 0,09 – 8 µg/l (mean 0.97 µg/l, N= 10).

OAEI (1996) reviews some results from the MISA (Municipal Industrial Strategy for Abatement) program in Ontario, Canada. Based on measurements from 9 different sites from October 1989 to September 1990 the concentration of DEHP in effluents from the organic manufacturing sector ranged from 0.4 to 19µg/l. The concentration of DEHP in the effluent from the inorganic chemical sector ranged from 0.22 to 65µg/l during the period 1989 to 1991. The average concentration of DEHP in effluents from the Petroleum refining sector was 1.9 µg/l (range 1.4 - 11 µg /l) based on monitoring data from 7 refineries during 1988 and 1989.

One of the producers reports a DEHP concentration of 334 µg/l from one measurement of effluent water from the production plant in February 2000. The DEHP concentration in the effluent water from their wastewater treatment system was at the same occasion 52 µg/l.

Sewage sludge

The Swedish EPA made a survey of organic chemicals in sewage sludge from 8 different municipal STPs with varying degree of industrial connection during 1987 (Swedish EPA, 1988).

The concentration of DEHP in the sludge ranged from 74 to 661 mg/kg dwt (mean 247 mg/kg dwt).

During 1989 – 1991 the Swedish EPA made another survey of organic chemicals in sewage sludge from 11 different municipal STPs. These samples were analysed by one laboratory and the concentrations ranged from 25 to 462 mg/kg dwt (mean = 174 mg/kg dwt) (Swedish EPA, 1992). In the same report additional data from 6 other STPs are reported. The analyses were made during the same period but by different laboratories. The concentrations of DEHP in these sludges ranged from 76 to 285 mg/kg dwt (mean = 144 mg/kg dwt).

Stockholm Vatten analysed sludge samples from two municipal STPs (Henriksdal and Bromma) in Stockholm, Sweden. The concentration in sludge from Henriksdal was 67 mg/kg dwt in 1989 (Stockholm Vatten, 1990) and 93 mg/kg dwt in 1991 (Stockholm Vatten, 1991a). The sludge from Bromma contained 116 mg/kg dwt in 1991 (Stockholm Vatten, 1991b).

In connection with a study on sludge application on agricultural soil, sludge was analysed for its contents of a large number of organic chemicals including DEHP (Henriksson, 1997). The sludge was collected from a STP in Malmö, Sweden. Samples were taken each year during the period 1991 – 1996 before the sludge was applied to the soil. The analyses were performed by two different laboratories using two different methods. The analyses from one laboratory gave a mean DEHP concentration of 105 mg /kg dwt (range 0 – 240) whereas the analyses from the other laboratory gave a mean of 49 mg/kg dwt (range 18 – 116).

Grüttner and Vikelsøe (1996), reported DEHP concentrations of 48, 45 and 47 mg/kg dwt from the Danish municipal STPs Avedøre, Skœvinge and Marselisborg, respectively. The samples were taken in October – November 1992. In July 1995 sludge from the same three STPs was analysed (Grüttner et al., 1995). In this study two other laboratories performed the analyses (the samples from Marselisborg were also analysed by the laboratory responsible for the 1992 analyses). The DEHP concentrations in sludge as analysed by one of the laboratories was 46, 44 and 43 for Avedøre, Skœvinge and Marselisborg, respectively, which is at the same level as in 1992. The results from the other laboratory indicated much lower concentrations (2.3, 0.9 and 1.7 mg/kg dwt) and are not considered reliable. The complementary analysis of Marselisborg sludge performed by the laboratory responsible for the 1992 analyses resulted in a DEHP concentration of 189 mg/kg dwt.

Kjølholt et al. (1995) compared the DEHP concentrations in winter and summer samples of sludge from three different Danish STPs (Herning, Skœvinge and Marselisborg). The winter samples were taken in February 1994 and the summer samples in June the same year. The winter sample from Herning contained 120 mg DEHP/kg dwt and the summer sample 38 mg/kg dwt. There were little difference between winter and summer samples from the other two STPs: Skœvinge, 17 mg/kg dwt (winter) and 18 mg/kg dwt (summer); Marselisborg 41 mg/kg dwt (winter) and 37 mg/kg dwt (summer).

Krogh et al. (1996), reports DEHP concentrations of 14 and 23 mg/kg dwt in samples taken late fall in 1995 from two Danish municipal STPs Herning and Ringkøbing, respectively.

In another Danish study (Kristensen et al., 1996**)** sewage sludge from 19 STPs representative for Danish sewage treatment were analysed. The samples were taken in 1994. The DEHP concentrations ranged from 4 to 170 mg/kg dwt with a mean concentration of 37.8 mg/kg dwt and a median conc. of 24.5 mg/kg dwt.

In a recent Danish study from the Århus area (Boutrup et al., 1998) sludge samples were taken from 6 different STPs during 1996 – 1997. The DEHP concentrations ranged from 9 – 49 mg/kg dwt with a mean of 25 mg/kg dwt.

Braaten (1996), reports results from analyses of DEHP in sludge from three Norwegian municipal STPs in 1996. The sludge from Bekkelaget close to Oslo contained 113 mg DEHP/kg dwt whereas the sludge from Fuglevik where there is a PVC-foil producer in the area contained 96 mg/kg dwt. Sludge from the third STP, Slemmestad (VEAS), contained 78.5 mg/kg dwt.

The DEHP concentrations in municipal sewage sludge from five different STPs in the Netherlands ranged from < 5 – 185 mg/kg dwt in samples taken in the period 1992 – 1993 (Bodar, 1997).

Furtmann (1996), reported data from a German survey from 1992. In sludge from a STP dominated by industrial effluents the DEHP concentrations were 40, 21 and 85 mg/kg dwt in fresh, activated and dewatered sludge, respectively in March. In June the DEHP concentrations were higher: 225 and 163 mg/kg dwt in fresh and activated sludge respectively. Concentrations of DEHP in sludge obtained in June 1992 from a STP predominated by household effluents were 194, 153 and 74 mg/kg dwt in fresh, processed and dewatered sludge, respectively.

Samples of digested sludge from 5 sewage treatment plants in Germany were analysed for DEHP (Kolb et al., 1997). DEHP was detected in all samples in concentrations ranging from 13.4-18.3 mg/kg dwt (mean = 16.3)

Webber and Lesage (1989) compared various methods for extracting organic compounds from municipal sludge. Out of six tested methods five gave recoveries of DEHP above 200% whereas one gave recoveries of 86 ± 72%. Sludge samples from 15 sites (mainly from industrial centres) were analysed with this method. DEHP was detected in 93% of the samples in concentrations ranging from 3 – 215 mg/kg dwt (median 80 mg/kg).

Webber and Nichols (1995) analysed sewage sludge from 11 locations across Canada. Sludge was sampled one day per month for six months, at each location, during the period of September 1993 to February 1994. The measured concentration in the sewage sludge had an overall mean of 163 mg/kg dwt.

3.1.4.2.1 Calculated PECs compared with monitoring data STP

Wastewater

In monitoring studies on different municipal STPs in Sweden, Denmark, Norway, and Germany measured concentrations in untreated wastewater (influent) varied between 4-250 µg/l. In the treated wastewater (effluent) the DEHP concentration varied between 0.07 and 28 µg/l with removal rates mostly in the range 90 – 99%. However, in a few cases removal rates were lower, in one case only 40%. For the scenario relevant to compare to effluents from municipal sewage treatment plants (life cycle stage 7 “Municipal STP”) the estimated DEHP concentration is 32 µg/l, which compares quite well with the highest measured concentrations.

There are very few data on concentrations of DEHP in purely industrial wastewater. In the material there is one example of very high DEHP concentrations (1,800 µg/l) in a tube that connects wastewater from an industrial area to the main inlet tube to a Swedish municipal STP. In effluent wastewater from industries and industrial areas in Europe, measured concentrations varied between 0.08 and 340 µg/l. The highest value was measured in wastewater from a leather

producing industry. Measured concentrations in the effluent are available from two DEHP producers. At Neste Oxo in Sweden, a DEHP concentration of 57 µg/l was measured in June 1990. Seven years later in November 1997 the measured effluent concentration of DEHP was measured to 0.08 µg/l. From another producer a measured value from February 2000 of 52 µg/l was reported. The effluent wastewater concentrations for the different local scenarios are calculated to be between 0 and 2.7 mg/l (see **Table 3.49**, **Table 3.51** and **Table 3.54**).

Sewage sludge

In monitoring studies on DEHP in municipal STP sludge the concentrations vary between 0 and 661 mg/kg dwt in sludge from Sweden, Denmark, Norway, the Netherlands and Germany. Two Canadian monitoring studies reported concentrations in the range of 33-440 mg/kg dwt. There are no measurements available on purely industrial sludge. The material gives no possibilities to draw conclusions about time trends for DEHP concentrations in sludge. Furthermore, it is difficult to reveal any big differences in DEHP concentrations in sludge from STPs with low or high rate of industrial connection. A crude overall mean for DEHP concentration in municipal sewage sludge is approximately 100 mg/kg dwt.

The calculated concentration in sewage sludge for the local municipal STP scenario (lifecycle stage 7a) is 909 mg/kg dwt. This is higher than the average measured value and also somewhat higher than the highest measured concentrations reported from municipal STPs.

3.1.4.3 Measured levels in the atmosphere

Available monitoring studies in precipitation and air are summarised in Tables 18 and 19, respectively, in Annex 1. DEHP has been found in the gas phase, solid phase (particles) and in the water phase (rainwater) of air samples. In some of the studies it is not clear which phases the concentrations reported takes into account.

In monitoring studies considered to represent regional scenarios, concentrations of DEHP between 0.3 and 300 ng/ m^3 were measured. The highest values were achieved on sampling sites in urban or unspecified polluted areas.

Thurén and Larsson (1990) measured DEHP in air by passing air through a glass fibre particle filter or polyurethane filters. The measurements were conducted at 14 stations spread over Sweden during 5 sampling periods (approximately 3 months) during 1984-1985. DEHP was found in concentrations ranging from 0.28 - 77.0 ng/m^3 (median 1.95). The levels of DEHP in air were positively correlated to the mean temperatures during the sampling periods at the different sampling stations. This is probably an effect of increased volatility (increased vapour pressure) that results in increased atmospheric residence time and susceptibility to long-range transport during warmer conditions. The overall conclusion was that no specific distribution pattern or gradients could be seen neither in air concentrations or in fallout, which implies that the import of DEHP from sources outside Sweden is small.

Measurements of DEHP and other phthalates in air have been performed by the Research Institute for Chromatography, RIC 1998-2000 (RIC, 2000a). Measured concentrations from four sites in the Netherlands ranged from 8-52 ng/m^3 (mean 28 ng/m^3, n=8) in winter and from 9 to 400 ng/m^3 (mean 118 ng/m^3, n=8) in summer. Air samples taken in Belgium varied from 0.5 to 34 ng/m^3 (mean 9.5 ng/m^3, n=11). The median concentration for all measurements was 19 ng/m^3 and the 90th percentile 54 ng/m^3. Close to a DEHP processing plant in Italy a concentration of 2295 ng/m^3 was measured.

In January 2001 air samples were taken at four sites in the Netherlands (RIC 2001b). The concentration of DEHP was below the detection limit of 2 ng/m^3 in all four samples. The low concentrations may be due to the low temperature at sampling (0°C) and the low traffic/ industrial activity (samples were taken on a Sunday).

In monitoring studies considered to represent continental or "background" levels DEHP concentrations in air ranged between 0.06 and 2.9 ng/m^3 (Ligocki et al., 1985; Atlas et al., 1988).

The rainwater concentrations and air deposition of DEHP was measured in several studies, summarised in Table 2 and 3 in Annex 1. In a German study (urban areas), DEHP is found in precipitation in concentrations between 60 and 9,820 ng/l (Furtmann, 1996). The overall mean measured concentration from four sites was 1,073 ng/l (n=105). Based on these data and a yearly precipitation the authors calculated an average annual deposition of 600 µg/m^2 corresponding to 1.8 µg/m^2/day.

Thurén and Larsson (1990) measured rainwater concentrations ranging from 8.3 to 429 ng/l with a mean value of 48 ng/l (n=48) at 14 sampling stations in Sweden. This was recalculated to an average deposition of 0.78 µg/m^2/d (range 1 – 9.4 µg/lm^2/d.

In Denmark the deposition rate on snow near a processing site was measured to be 0.7-4.8 µg/m^2/day (Lökke and Rasmussen, 1983). In another Danish study 0.57-2.54 µg/m^2 · day was detected (Vikelsøe et. al., 1999). In studies in US the deposition of particle bound DEHP was estimated to 1.2 and 6.75 µg/m^2/day in two different studies (Atlas et. al., 1988). These data are assumed to represent a regional/continental exposure scenario. Based on these data an average deposition rate of 1 µg/m^2/d = 1 · 10^{-9} kg/m^2 · day is chosen for calculation of total deposition in EU (see Section 3.1.6.3.1 below).

Dry particle bound deposition and wet deposition is probably covered by the monitoring studies. Dry gaseous deposition, however, is not dependent on settling on a horizontal surface. The proportions between gaseous and wet particle/bound deposition is not known. However, considerable amounts of DEHP do occur in the gaseous phase, about 30-60% according to BASF (1989), which indicate that reported air deposition rates are underestimations.

In other studies, performed mainly in rural or remote marine areas in North America and Sweden, the concentrations in rainwater ranged between 0.001 and 618 ng/l.

3.1.4.3.1 Calculated PECs compared with monitoring data Atmosphere

In monitoring studies considered to represent continental or "background" levels DEHP concentrations in air ranged between 0.06and 2.9 ng/m^3, to be compared with the calculated continental PEC of 1.6 ng/m^3.

The calculated regional concentration in air is 7.6ng/m^3. This is lower than measured concentrations from The Netherlands and Belgium, median 19 ng/m^3 and 90th percentile 54 ng/m^3, assumed to represent regional concentrations (RIC, 2000a). It is also lower than some of the measurements performed in Sweden at sampling sites in urban areas (Thurén and Larsson (1990).

The highest calculated PEC_{air} for processing scenarios is 2,100ng/m^3. This compares quite well with the only measured value 2,295 ng/m^3 close to a DEHP processing plant in Italy (RIC, 2000a).

The dry particle bound and wet deposition within EU can be estimated to 1,300 tpa (see below). Since the dry gaseous deposition is not included in this value the total air deposition within EU will probably be higher.

- Continental deposition rate (see 3.1.6.3): $1 \cdot 10^{-9}$ kg/m^2 · day
- Total area of EU (TGD): $3.56 \cdot 10^6$ km^2 = $3.56 \cdot 10^{12}$ m^2
- EU deposition: Rate · Area = $1 \cdot 10^{-9} \cdot 3.56 \cdot 10^{12}$ = 3,560 kg/day = 1,300 tpa

It is estimated that about 536 tpa is released to air (**Table 3.49**, all release scenarios included). The deposited amount calculated above is more than twice as high as the emitted amount. With a half-life of 1 day in the atmosphere and a residence time of 6.4 (continental) this difference will be even larger. In the EUSES model a median concentration of 19 ng/m^3 which is the median derived from the RIC study corresponds to a total release to air of about 3,600 tpa .

Even though the database is small it seems from the available monitoring data and deposition rates that the EUSES model slightly underestimates the regional air concentration. There may be several reasons for this discrepancy:

1. The emission rates to air may be higher than estimated.
2. The emitting surface area may be higher than estimated
3. Underestimation of the distribution to the atmospheric compartment of waste remaining in the environment (dust particles).
4. The degradation rate in the atmosphere may be lower than estimated.
5. The atmospheric residential time (TGD default) may be longer than estimated.
6. Selected monitoring values may not be representative for a regional concentration.

As a consequence, the measured median value, 19 ng/m^3, is used to override the calculated regional PEC_{air}.

3.1.4.4 Measured levels in the terrestrial compartment

Available monitoring studies in soil are summarised in Table 20, Annex 1. The majority of the studies concern agricultural soil.

The concentration of DEHP in soil was measured during use of STP sludge as a fertiliser. In a study from Switzerland (Diercxsens and Tarradellas, 1987, in Bergkvist P. et. al. 1989, Naturvårdsverket 1992), the soil concentration was 0.12-0.19 mg/kg dwt directly after application and < 0.02 mg/kg dwt after one month.

Concentrations of 4.2-139 mg/kg dwt was found in compost consisting of 50-75% organic domestic waste (Kjölholt et al, 1998).

In a long-term study from West Germany with extremely high doses of STP sludge (333 tonnes dwt/annum) the concentration was slightly more than 5 mg/kg dwt after 10 years application (Kampe, 1987, in Bergkvist et. al. 1989). The measured concentration in the control was 0.024 mg/kg dwt.

In Canadian study, the soil concentration was measured in 10 "typical" agricultural soils (Webber and Wang, 1995). The concentration varied between 0.08 and 2.7 mg/kg dwt, mean 0.42 mg/kg dwt.

Concentrations of DEHP and other phthalates in Dutch soil samples were analysed by ALcontrol (1999). Single or duplicate soil samples were taken from approximately 30 sites, including one airport site, different distances from roads and motorways, etc within the Netherlands. The samples were extracted with dichloromethane and the analyses were carried out on an LC-LVI-GCMS. Measures were taken to prevent contamination of the samples prior to analysis. The detection limit was 0.025 mg/kg dwt.

The levels of DEHP in samples which could not be attributed to local emission sources ranged from < 0,025 and 0.17 mg/kg dwt (mean 0.057 mg/kg dwt; 90th percentile 0.11 mg/kg dwt; n= 33). No clear gradient could be seen at different distances from motorways.

In a study by Vikelsøe et al. (1999) an agricultural soil, heavily amended with sewage (17 tonnes dwt/ha · year) sludge for 25 years, was analysed down to a depth of 60 cm 6 years after the sludge use had ceased. Among several xenobiotics DEHP and DINP were determined. The results are presented in **Table 3.57**.

Table 3.57 DEHP concentration in soil after amendment with sewage sludge

	Depth (cm)	DEHP (µg/kg dwt)	DINP (µg/kg dwt)
Agricultural soil, amended with high amounts of sewage sludge for 25 years, changed to artificial fertiliser 6 years before sampling, cattle grazing	0-10	990	130
	10-20	1,700	220
	20-30	1,400	200
	30-40	880	96
	40-50	590	93
Same location, sampled 2 years later	0-10	1,400	410
	10-20	1,700	540
	20-30	1,800	670
	30-40	3,400	910
	40-50	1,200	280
	50-60	550	63

These results indicate that DEHP and DINP are very slowly degraded (or that the deposition is high). The study also indicates that both substances can migrate to deeper soil layers. For DEHP the author suggest a downward movement of 10 cm/year. This phenomenon might be explained by leaching of particulate matter. In parallel also a run-off zone of a meadow was analysed. In contrast to the agricultural soil DEHP here stayed in top layer. The authors assume that higher ground water level and no mixing by ploughing cause this difference. The occurrence of DEHP was strongly correlated to the clay content of the soil. In the sand layers DEHP seems to be eluted. The authors concluded that this observation give concern for risk of ground water contamination.

3.1.4.4.1 Calculated PECs compared with monitoring data Terrestrial compartment

In 10 "typical" agricultural soils in Canada (Webber and Wang, 1995) which were not treated with STP sludge, the mean measured DEHP concentration was 0.42 mg/kg dwt, to be compared with the regional calculated PEC for agricultural soils, 0.07 mg/kg dwt.

In 30 soils in the Netherlands (ALcontrol, 1999), attributed mainly to air deposition of DEHP measured levels ranged from < 0.025 to 0.17 mg/kg dwt. These data may be compared with the calculated regional PEC for natural soils 0.015 mg/kg dwt.

In a long-term study from West Germany with extremely high doses of STP sludge (333 tonnes dwt/annum) a concentration of slightly more than 5 mg/kg dwt after 10 years of application was measured. In a Danish soil fertilised with sludge for 25 years the DEHP concentration in the upper layers was around 1 mg/kg dwt 6 years after sludge fertilising had ceased. This can be compared to the estimated concentrations in agricultural soil for the different polymer and non-polymer scenarios that range between 0.5 and 103 mg/kg.

Although there is a limited amount of measured data available these indicate that the regional concentrations in natural and agricultural soil may be underestimated by EUSES. On the other hand, some of the calculated local PECs seem to be overestimated.

3.1.4.5 Measured levels in biota

A large number of predominantly older data, from the seventies and eighties, on DEHP in biota are available in the open literature. Most of the studies have not been submitted by industry but are cited in one or several reviews. For the compilation of this section some but not all original publications have been evaluated separately. Instead available reviews from e.g. authorities in the USA and UK and others have been used. Monitoring studies in biota referred to in the text are summarised in Table 21, Annex 1.

Most studies are concerned with aquatic invertebrates and fish but there are also a few data on DEHP in plants, terrestrial arthropods and single data on other aquatic vertebrates such as tadpoles and seal.

This review should ideally give an overview of DEHP levels in biota with respect to taxonomic groups, local and regional exposure regimes and possible time trends. Of the taxonomic groups primarily aquatic invertebrates (e.g. arthropods, molluscs) and fish are covered. Where possible data considered being more representative of local exposure in comparison to regional or background concentrations have been identified. With regard to time trends there are hardly any data collected in a systematic way over time to allow an analysis of temporal variability.

<u>Plants</u>

A recent study (Elf Atochem, 1997) on phthalates in the Seine estuary in France reported DEHP at 650 µg/kg (dwt) in the green macroalgae *Enteromorpha* sampled at only one station.

Japanese data are cited to report 63,000 µg/kg in plankton in 1981 whereas no DEHP were detected in plankton from brackish water in an industrial area in Finland in the late seventies. It should be noted that plankton is composed not only of phytoplankton but also of zooplankton and detritus etc.

German data from the early to mid 1980-ies on reeds and grasses from the River Elbe (at Hueckenlock and Harburger Hafen) report DEHP in concentrations of 2,300 to 11,300 µg/kg (dwt). Grass from the vicinity of a PVC-processing plant without exhaust air cleaning in Niedersachsen contained 1,200-2,500 µg/kg (dwt).

In the terrestrial plant yarrow (*Achillea millefolium*) from a Danish production site for plastcisers DEHP levels ranged from < 20 to 65 µg/m^2 leaf surface.

Invertebrates

Data from the seventies report DEHP in aquatic arthropods at 100 µg/kg (dwt) and 200 µg/kg, respectively, from an industrial area in Finland and a single sample of dragonfly naiads from a fish hatchery in the USA.

In a study in two Swedish rivers in the mid eighties aquatic invertebrates were collected upstream and downstream of industrial effluent discharge points. Organisms from the river Ronnebyån taken upstream the discharge (1-5 km) contained 310-14,400 µg/kg (WWT). Organisms were absent at the discharge point. Organisms from the river Svartån taken upstream the discharge (1-8 km) contained 110-1,810 µg/kg (WWT) while the concentration in dragon fly larvae (*Odonata sp*) at the discharge was 5,310 µg/kg (WWT).

German data from the early to mid eighties on gammarids in the River Elbe report DEHP in concentrations from Hueckenlock at 300 µg/kg (dwt) and Harburger Hafen at 800-1,100 µg/kg (dwt). Tubifids from the same areas contained 200-300 µg/kg (dwt) and 500-900 µg/kg (dwt), respectively.

In two samples of molluscs from the River Elbe, Germany the DEHP levels were 2,300 and 4,300 µg/kg (dwt). Mussels collected in the early eighties from the river Crouch, Essex in the UK showed levels of 9-214 µg/kg (WWT) in the digestive gland. DEHP in mussels from the Seine estuary in France sampled in 1997 ranged from 1,390 to 1,850 µg/kg (dwt).

Samples of molluscs and mixed invertebrates (species not specified) were taken from three locations in the Netherlands in November 2000 and analysed for phthalates including DEHP (RIC 2001c). The DEHP concentration in the three mollusc samples was 185, 194 and 624 µg/kg (WWT), respectively. The corresponding dry weight concentrations were 1,188, 996 and 4,039 µg/kg. The mixed invertebrate samples had DEHP concentrations of 1,221, 1,492 and 1,546 µg/kg (WWT), respectively. The corresponding dry weight concentrations were 9,270, 18,508 and 11,621 µg/kg.

The only data available on DEHP in terrestrial invertebrates report 2,800 µg/kg (dwt) in soil arthropods from an industrial area in Finland in the late seventies.

Vertebrates

Data on DEHP in the USA from the early seventies report concentrations in tadpoles from a fish hatchery in Iowa at 300 µg/kg. Further data from the same study on DEHP in fish from agricultural and industrial areas in North America ranged from not detected to 3,200 µg/kg.

In a survey of fish from Canadian lakes and rivers a background level of DEHP in the samples at 15 µg/kg (WWT) was determined to be due to laboratory contamination by analysing blank samples. Levels less than two times the background were recorded as trace. DEHP was found at 104 µg/kg (WWT) in eel, while trace levels were found in catfish and pickerel.

Levels of DEHP in various aquatic biota, mainly fish, collected from the Gulf of Mexico in the early seventies varied from 1 to 135 µg/kg (WWT).

Several surveys of DEHP in fish collected in Japan during the seventies report concentrations at 70-450 µg/kg, 40-720 µg/kg, 10-19,000 µg/kg (mean 290 µg/kg), and 50-1,800 µg/kg.

Fish from an industrial area in Finland collected in the late seventies contained DEHP from 100 µg/kg (dwt) in fry and sticklebacks to 1,100 µg/kg (dwt) in roach muscle and 2,300 µg/kg (dwt) in pike liver.

Several species of fish collected in the late seventies from the Gulf of St. Lawrence and the Bay of Fundy in Canada were analysed for DEHP in lipid extracts. Levels on fresh weight basis were found to range from not detected in plaice and redfish up to 7,200 µg/kg (WWT) in herring muscle. Mackerel muscle and cod muscle contained 6,500 and 5,200 µg/kg (WWT), respectively. Eel had lower levels of 370 µg/kg (WWT).

A maximum DEHP concentration in unspecified shark samples has been cited at 7,100 µg/kg.

Concentrations of DEHP in fish liver and muscle (dab, plaice and whiting) collected in the early eighties from Tees Bay, UK were 43-86 µg/kg (WWT) and 13-51 µg/kg (WWT), respectively. Dab from the Crouch estuary in Essex, UK showed lower levels in liver, approximately 2 µg/kg (WWT) and in muscle 14 µg/kg (WWT).

German data from several surveys of various species of fish from the early to mid 1980-ies from the lower Rhein (Niederrhein) and Elbe (Süderelbe) rivers showed concentrations of 17-70 µg/kg (WWT) and mean concentrations of two samples of five bream were 300 and 500 µg/kg (WWT), respectively. Mixed fish samples of unspecified origin contained < 500 µg/kg (WWT).

In a survey of plasticisers in fresh water fish in Austria in 1997 a total of 180 fishes were collected at 58 locations (Pfannhauser et al. 1997). The samples where collected during 2 months from March to May. Sampled species were rainbow trout, brown trout, char, carp and eel. Samples of dorsal muscle free of skin and bones were taken for the determinations. DEHP was the only plasticiser found in all of the 71 (39.4%) positive samples containing measurable amounts of phthalates. The highest level of DEHP found was 2,600 µg/kg (WWT) in carp. At five sites DEHP levels in a total of eight fish samples exceeded 1,000 µg/kg (WWT). The 90th percentile was approximately 500 µg/kg (WWT).

In a survey in The Netherlands (RIC 2000b and c) 25 fish samples (roach and bream) caught during 1998 at 18 different locations were analysed for phthalates. The DEHP concentrations ranged from < 1 µg/kg to 149 µg/kg (WWT) with a 90th percentile of 89 µg/kg WWT. These values are lower than those from the Austrian study. However, due to the fact that fewer samples were taken and that the samples were stored for two years prior to analysis these values are considered less reliable. In a complementary study (RIC 2001d) three additional fish samples (roach) caught in autumn 2000 were analysed. The DEHP concentration was approximately 300 µg/kg (WWT) in all three samples. One sample was taken from a location sampled also in the previous study. The DEHP concentration in the previous sample was much lower, 20 µg/kg WWT.

In two hatchery-reared juvenile salmon (S*almo salar*) in Canada levels of DEHP at 12,900 and 16,400 µg/kg (lipid weight) were measured in the early seventies. In the same study blubber from a common seal pup (*Phoca vitulina*) contained 10,600 µg/kg (lipid weight). No collection site was stated for the seal.

3.1.4.5.1 Calculated PECs compared with monitoring data Biota

PECorals in aquatic biota for the exposure of predators have been calculated for fish, mussels, amphipods and zooplankton. According to the TGD and EUSES PECorals are calculated as the result of equal contribution to exposure from the local and regional levels. This has been taken into account in the PECorals for production on the local scale given in **Table 3.50** and for other local scenarios given in **Table 3.53** and **Table 3.55**. Local aquatic PECorals for DEHP vary between 2 and 229 mg/kg (WWT). The regional aquatic PECorals vary from 1.8 to 6 mg/kg (WWT).

Measured levels are available for fish, molluscs and aquatic arthropods although the quality of data and the level of exposure they represent are in many cases difficult to assess. The data on aquatic arthropods include amphipods and are taken to correspond to amphipods in the calculated data. The measured data on plankton are too sparse and vary too much to allow any attempt of comparison to the calculated values. There are no data for comparison to the calculated levels in earthworms.

Measured levels of DEHP in aquatic arthropods vary between 100 µg/kg (dwt) and 14,400 µg/kg (wet weight). The highest value comes from the freshwater isopod *Asellus aquaticus* collected several kilometres upstream a known industrial discharge. The upper end of this range agrees fairly well with the calculated level for the regional environment of 6 mg/kg WWT. The highest measured value from a local discharge site is 5,300 µg/kg (wet weight) in dragonfly larvae. Recent data on "mixed invertebrates" from the Netherlands range from 996 to 4,039 µg/kg WWT. The calculated local levels (6 –8 mg/kg WWT) are similar to that.

Measured levels of DEHP in molluscs vary between 10 µg/kg (wet weight) and 4,300 µg/kg (wet weight). No reviewer has specifically stated that the reported values represent local exposure levels. The molluscs showing the highest values were collected in the River Elbe and are assumed to represent regional exposure. The highest values agree fairly well with the calculated regional concentration of 5.5 mg/kg WWT. Local calculated concentrations are in many cases similar to these levels but some are one to two orders of magnitude higher. Generic production sites based on default values are much higher.

Measured levels of DEHP in fish vary between a few µg/kg and 19,000 µg/kg. Several studies report DEHP in the range of 2,600 to 7,200 µg/kg on a fresh weight basis in muscle. The most recent study from Austria in 1997 reported a maximum value of 2,600 µg/kg (WWT). At five Austrian sites DEHP levels in a total of eight fish samples exceeded 1,000 µg/kg (WWT). The 90th percentile is 250 -500 µg/kg (WWT). In the recent Dutch surveys the concentrations ranged from below detection limit 1µg/kg (WWT) to 300µg/kg (WWT). None of the studies are explicitly referred to as representing local exposure levels. This range of measured values corresponds well with calculated regional levels of approximately 2 mg/kg and also with most of the local PECorals in fish.

In summary, for three out of four trophic levels for which PECoral aquatic have been calculated there are measured levels for comparison in the range from approximately 1-10 mg/kg (WWT). From the available information it is assumed that these data generally represent regional or continental exposure rather than TGD local exposure scenarios. The upper end of the range of measured values agrees fairly well with calculated regional values. In a number of local scenarios the PECoral aquatic exceed the measured values with one to several orders of magnitude. However, in the cases where the difference is most pronounced calculated values are based on default values.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

3.2.1 Aquatic compartment

The very low water solubility of DEHP causes problems when testing toxicity to aquatic organisms and when interpreting the results. Most aquatic studies with DEHP have been made at test levels, which exceed the "molecular" solubility of approximately 3 µg/l (see Section 0). However, stable dispersions are formed up to levels of around 300 µg/l. DEHP readily adsorbs to organic particles and also to various surfaces. In test solutions with concentrations higher than the water solubility, emulsions of micro-droplets of DEHP and surface films may be formed (Pedersen et. al. 1996). This may cause unstable test solutions where the bioavailable fraction is lower than the nominal concentration, and the exposure of the organism cannot be correctly quantified. Formation of micro-droplets or surface films may also contribute to effects by direct physical interference, e.g. entrapment at the surface (flotation) or obstruction of the gas flow over the gills (Pedersen et al. 1996). Judging from toxicity tests with Daphnids where problems with solubility have been reported, the "apparent water solubility" in the tests seems to be roughly in the order of 0.1 mg/l. Above this approximate level test solutions seem not to be stable and solubility-related problems start to arise e.g. floaters.

In the environment, DEHP is likely to be sorbed to any suspended particles in natural waters and the presence of dissolved organic material may furthermore increase the apparent water solubility. This may lead to a higher apparent water solubility of DEHP than predicted from the physico-chemical properties (Pedersen et al. 1996). The adsorption of lipophilic substances onto particles and colloids may either decrease or increase the bioavailability and thus the toxicity. For most substances, particles and colloids probably decrease the bioavailability, but for certain types of organisms (especially suspension feeders or detritivores) the reverse fact might be true (Lundberg 1994). As DEHP tends to accumulate in sediments, the evaluation of toxicity to organisms living in or on sediment is essential.

Sediments may act as a sink for, and as a source of chemicals in the aquatic compartment. Sediments integrate the effects of surface water contamination over time and space and may thus present a hazard to aquatic communities (both pelagic and benthic), which is not directly predictable from concentrations in the water column (TGD, 1996).

Several insect larvae spend their larval stage on/in the sediments, and detritivorous species, such as several chironomids, also feed from the decaying material in the sediments. Therefore effects on benthic organisms are of concern, not only as threats to individual species as such, but because they play an important roll in the recycling of detrital material.

However, to date only few tests on sediment organisms have been conducted in Europe with existing substances. The sediment environment is very complex, consisting of a quasi-stable physical system in which numerous physico-chemical and micro-biological gradients exist and interact. Inorganic and organic substances of both natural and anthropogenic origin partition between sediments, interstitial water, overlaying water, and resident biota. Many sampling and laboratory manipulations can have a dramatic impact on partitioning and, thereby, affect toxicity responses in the test species (Burton et. al., 1992). The selection of representative organisms and the selection of standardised sediments are still under discussion.

The high lipophilicity of DEHP implies that toxicity studies involving sediment-dwelling organisms are of special interest in the present risk assessment. Some standardised tests and

some papers published in scientific journals exist for DEHP and sediment exposed organisms. These papers show somewhat contradictory results and some 'critical', controversial, non-standardised sediment studies have therefore been paid special attention in the survey below.

3.2.1.1 Toxicity to fish

The toxicity of DEHP to fish has been tested in short and long-term studies of varying quality. Both static, semistatic and flow-through test regimes have been applied. The problems that arises when testing the aquatic toxicity of a poorly water soluble, lipophilic substance like DEHP, as discussed in Section 3.2.1, are of course also obvious when the test organism is fish. In many of the tests there has been problems maintaining test concentrations. In studies where both nominal and measured test concentrations are reported the measured concentrations are often lower. Therefore studies where only nominal test concentrations are reported may underestimate the toxicity of DEHP. The occurrence of physical effects such as e.g. obstruction of gas flow over the gills is not unequivocally supported by the short-term toxicity studies. Concentrations up to 3,000 mg/l have been tested without causing mortality except for one study where a LC_{50} of 540 mg/l is reported for rainbow trout (Hrudey et al., 1976). The mortality in this study is assumed by the authors to be caused by physical action. In the long-term studies exposure of 0.554 mg DEHP/l for 168 days did cause slight effects on growth but no mortality in Japanese medaka (Defoe et al., 1990).

Exposure via water

Several studies on the short-term toxicity of DEHP to several fish species have been performed using static, semistatic, and flow-through test-systems. Concentrations up to 3,000 mg/l have been tested and in most of the studies no mortality or other effects are seen (see **Table 3.71**). One study (Hrudey et al., 1976) reports a 96-hour LC_{50}-value of 540 mg/l for rainbow trout (*Onchorhynchus mykiss*). This value is based on the nominal exposure concentration. In this static study fingerling rainbow trout were exposed to DEHP emulsified in the water without the use of any carrying agents or stabilisers. The authors postulate that the test emulsions may have killed primarily by means of a physical coating action on the fish. Dead fish were coated possibly by the test compound, and fish were observed to stay near the surface and cough throughout the test. It shall be noted that, in several other studies higher exposure concentrations have been used without causing mortality.

From the short-term toxicity studies it can be concluded that DEHP has no acute effect on fish at concentrations far exceeding its water solubility.

The long-term toxicity of DEHP to juvenile and adult fish of different species has also been studied. The results from these studies are summarised in **Table 3.72**.

Mehrle and Mayer (1976) exposed adult, 7.5 month old, fathead minnows (*Pimephales promelas*) continuously for 56 days at 25°C to DEHP. The measured exposure concentrations ranged from 1.9 to 62 µg/l. Acetone was used as a carrier solvent in concentrations not exceeding 0.25 ml/l. It is not clearly stated if acetone was used in the control. In this study, which was basically a bioaccumulation study, DEHP had no effect on growth or mortality at concentrations up to 62 µg/l.

Defoe et al. (1990) used juvenile Japanese medaka (*Oryzias latipes*), < 1 to 3 days old, to study chronic effects of DEHP. The fish were exposed for 168 days to a mean measured DEHP concentration of 0.554 mg/l in a flow through test. No solvent or dispersing agent was used. This exposure had no significant effect on survival. However, the weight of DEHP exposed fish was

reduced 13% compared to control after 168 days of exposure. This reduction was statistically significant ($p < 0.05$). However, since only one concentration was tested, the weight reduction was rather small, and the exposure concentration was above the apparent water solubility of DEHP it is not considered suitable to derive a NOEC from this study.

Adema et al. (1981) reports a NOEC > 0.32 mg DEHP/l based on nominal concentrations from a study were 3-4 week-old guppy (*Poecilia reticulata*) were exposed to DEHP for 28 days. DMSO 0.1ml/l was used as solvent.

Mayer et al. (1977) studied the effects of several organic chemicals including DEHP on the synthesis of vertebral collagen and hydroxyproline. Three fish species were used: brook trout (*Salvelinus fontinalis*), fathead minnow (*Pimephales promelas*) and rainbow trout (*Onchorhynchus mykiss*). Adult brook trout (1.5 years at initiation of exposure) were continuously exposed to DEHP concentrations ranging from 0 - 52 µg/l for 150 days. The fathead minnows were 10 day old at initiation of exposure and were continuously exposed to concentrations ranging from 0 - 100 µg/l for 127 days. In the rainbow trout study eyed eggs were continuously exposed from 10 days before hatching to 80 days post hatch to DEHP concentrations ranging from 0 - 54 µg/l. In all studies acetone was used as a solvent. However, no information about the acetone concentrations used is given. In the brook trout study the collagen content in backbone was significantly reduced and the level of hydroxyproline in collagen was significantly increased ($p = 0.05$) already at the lowest DEHP exposure (3.7 µg/l). However, there was no dose response relationship and the level of reduction/increase was approximately the same at all exposure levels. Also in the fathead minnow study the collagen content of backbone was significantly reduced and the hydroxyproline content in collagen significantly increased at the lowest DEHP exposure (11 µg/l). The reduction in collagen content was dose dependent but the increase in hydroxyproline was not. In the embryo-larval study with rainbow trout collagen content was significantly reduced at next lowest exposure level, 14 µg/l, whereas hydroxyproline content did not differ significantly from the control. There was no mortality and no significant effects on growth in any of the three fish species tested. Although this study gives very low NOEC-values regarding these biochemical parameters it is not considered relevant to use them in the risk assessment since the exposure did not result in any effects on growth or survival.

There are also several embryo larval studies of shorter or longer duration with different fish species in which fertilised eggs and resulting fry have been exposed for DEHP. These studies are summarised in **Table 3.73**.

Birge et al. (1978 and 1979) performed semistatic and flow through embryo larval studies with 5 fish species. The tested substance, reported as DOP, was actually DEHP according to the Canadian water criteria guidelines (1993).

In the semistatic studies (Birge et al. 1978) fertilised eggs from channel catfish (*Ictalurus punctatus)* and redearsunfish (*Lepomis microlupus)* were exposed for 3 - 4 days before hatch until 4 days post hatch to 5 nominal exposure concentrations ranging from 0.01 - 100 mg/l. Acetone was used as carrier solvent, 0.04 - 4 mg/l depending on toxicant concentration. Controls with and without solvent were maintained simultaneously. It is not clear if each test concentration was compared to a control with similar solvent volume. This put some question marks to these studies. For channel catfish the nominal LC_{50} was1.21 mg/l at hatch and 0.69 mg/l 4 days post hatch. Corresponding LC_{50}-values for redear sunfish was 77.2 mg/l at hatch and 6.18 mg/l 4 days post hatch. No NOEC-values are given in this study. For channel catfish (4 days post hatch) the mortality was 10% at a nominal concentration of 0.1 mg/l and 45% at 1 mg/l. For redear sunfish the corresponding mortality was 0% and 12%. As effects were seen

only at concentrations well above the "true" water solubility and at or above the 'apparent' water solubility, it is uncertain if the effects seen are due to the intrinsic toxicity of DEHP. It is therefore not considered appropriate to use these results in the risk assessment.

In the flow through studies (Birge et al., 1978 and 1979), fertilised eggs from largemouth bass (*Micropterus salmoides*) and goldfish (*Carassius auratus*) were exposed for 3 - 4 days before hatch until 4 days post hatch. Eggs from rainbow trout (*Onchorhynchus mykiss*) were exposed for 22 days pre hatch until 4 days post hatch. The tests were run at two water hardness levels, 50 mg $CaCO_3$/l and 200 mg $CaCO_3$/l. No carrier solvent was used.

The resulting LC_{50}-values based on measured exposure concentrations are shown in **Table 3.58**.

Table 3.58 LC_{50}-values based on measured DEHP exposure concentrations

Species	LC_{50} at hatch (mg/l)		LC_{50} 4 days post hatch (mg/l)	
	50 mg CaCO3/l	200 mg CaCO3/l	50 mg CaCO3/l	200 mg CaCO3/l
Largemouth bass	65.5	32.1	55.7	45.5
*	63.9	66.1	42.1	32.9
Goldfish	> 186	> 191	> 186	> 191
Rainbow trout	139.1	154.0	139.5	149.2

* Results from the same study according to Birge 1978

No NOEC-values are given in the report. We do not consider it relevant to specify NOECs for the purpose of this risk assessment. The reason for this is that there is little or no mortality at test concentrations at or below the apparent solubility of DEHP (approximately 0.1 mg/l) for these species. Mortality exceeding 10% only occurs at concentrations far exceeding the true water solubility (see **Table 3.59** below).

Table 3.59 Mortality at different DEHP exposure concentrations

Species	50 mg CaCO3/l exposure conc. (mg/l)	Mortality 4d post hatch (%)	200 mg CaCO3/ l exposure conc. (mg/l)	Mortality 4d post hatch (%)
Largemouth bass	0.055	5	0.065	4
	0.3	9	0.3	10
	46.3	33	35.5	36
Goldfish	0.52	1	0.44	6
	28.1	14	40	21
Rainbow trout	0.48	5	0.5	10
	55.3	9	48.9	16

Mehrle and Mayer (1976) used rainbow trout eggs to study the effects of DEHP. The eggs were continuously exposed to DEHP at concentrations averaging 0, 5, 14 and 54 µg/l for 12 days before hatch and the resulting fry for 90 days after hatch. Acetone was used as carrier solvent at a concentration not exceeding 0.28 ml/l. There was no increase in egg mortality or any effects on hatchability in the DEHP exposed groups compared to control. There was however an increase in the mortality of sac fry within 5 days after hatching in the two highest concentrations. The mortality at the exposure levels 14 and 54 µg/l was 15 and 9% respectively compared to 2% in

the control. This increase in mortality was statistically significant ($p < 0.05$) and increased to 20 and 23% compared to 6% in the control 24 days after hatch. Thereafter there were no statistically significant differences in mortality or growth between DEHP exposed fish and the controls for the remainder of the exposure period. The dose response relationship in this study was weak and the acetone concentration used was too high. OECD-guideline 210 recommends a concentration of solubilising agents not greater than 0.1 ml/l. Furthermore, the NOEC obtained in this study was far below that in other studies with rainbow trout (Defoe et. al (1990), Mayer et. al (1977), Birge et. al (1979)). The results from this study are therefore not considered relevant for the purpose of risk assessment. It can also be noted that the results have been determined as unacceptable by the USEPA after personal communication with the authors of the study.

In another study Defoe et al (1990) exposed rainbow trout eggs continuously to 5 concentrations of DEHP. The measured concentrations ranged from 48 to 502 µg/l. No carrier solvent was used. The exposure started < 72 hours after fertilisation and continued for a total of 90 days (50 eggs/aquarium, 2 aquarium/conc.). After three weeks 15 eyed embryos were selected for the survival and growth part of the study. No statistically significant effects on hatchability, survival or wet weights were seen in any of the test concentrations. However, mean wet weights were reduced by 9.7 and 10.2% compared to control weights in the two highest exposure concentrations (259 and 502 µg/l) at the end of the study. This difference, although not statistically significant, may be an indication of effects on growth (see juvenile Japanese medaka study by Defoe et al where a small but statistically significant weight reduction of 13% was recorded).

In a preliminary range finding study Rhodes and McAllister (1990) exposed rainbow trout eggs from approximately 24 h pre hatch until 18 days post hatch. The exposure was performed in a flow through system and no carrier solvent was used. The mean measured DEHP concentration at the highest exposure level was 0.97 µg/l, which was 2.4% of the nominal concentration (40 µg/l). No effects on hatchability or survival were observed at or below this exposure concentration. In the definitive investigation Cohle and Stratton (1992) used newly fertilised rainbow trout eggs (fertilised < 8 hours before study initiation). The eggs were exposed for approximately 35 days prior to hatch and 35 days post hatch to mean measured DEHP concentrations of 0.33, 0.89, 2.6, 4.6 and 7.3 µg/l. No statistically significant reductions in hatchability or survival were indicated at any test level. A statistically significant reduction in weight and length was indicated at the next lowest exposure concentration but not in any other of the exposure concentrations. This finding is therefore not considered substance related.

Mayer et al. (1977) noted no mortality or effects on growth when eyed eggs of rainbow trout were exposed from 10 days before hatch until 80 days post hatch to DEHP concentrations ranging from 0 to 54 µg/l (see also section on long term exposure above).

Adema et al. (1981) reports a nominal NOEC > 0.32 mg DEHP/l for mortality, egg development and growth from embryo larval studies with flagfish (*Jordanella floridae*) and Japanese medaka (*Oryzias latipes*). Eggs were exposed to DEHP < 36 hours after fertilisation until a maximum of 28 days after hatch in a semistatic test system (test solutions changed 3 times/week). DMSO 0.1ml/l was used as solvent. In a similar embryo larval study with zebra fish (*Brachydanio rerio*) Canton et al. (1984) reports a NOEC > 1mg DEHP/l for mortality, egg development and growth. Van den Dikkenberg et al (1989) used the same method when they studied the usefulness of the Three-spined stickleback (*Gastrosteus aculeatus*) as a test organism. The reported NOEC for this species was > 0.32 mg/l.

Chikae et al. (2004a) studied the effects of DEHP on embryos of Japanese medaka (*Oryzias Latipes*). Newly fertilised eggs were incubated in a semistatic system and exposed to the nominal

concentrations 0, 0.01, 0.1, 1.0 and 10.0 µg DEHP/l (40 eggs/group) until hatching. Ethanol was used as solvent (< 100 µg/l). Eyeing, hatching time and hatching success were studied. After hatch, the fry (n = 25 – 43) were transferred to a post-hatch solution for growth and for 5-6 months. Mortality and body weight were recorded as well as sex ratio and gonadosomatic index for the surviving fish. No effects were seen on eyeing or hatching success. Significantly delayed hatching were observed at 0.1 and 1.0 µg DEHP/l, but not at 10.0 µg DEHP/l. Mortality was significantly higher in the 0.01, 0.1 and 1.0 µg DEHP/l groups, although not in the 10.0 µg DEHP/l group. Sex ratio was significantly changed towards females at 0.01 µg DEHP/l. Whether this was caused by feminisation or by male specific lethality could not be determined. Body weights of males decreased in a dose dependent manner and were significantly lower ($\leq$ 25%) at the three highest concentrations, 0.1, 1 and 10 µg DEHP/l respectively. Bodyweights of exposed females were not significantly different from the control. No significant effects on gonadosomatic index neither for male nor female medakas could be seen. The only dose-related effects that could be seen in this study was the decreased body weight in males. However, dose-related effects on body weights has been not been observed in any other studies on DEHP. The interpretation of the study is hampered by the high and variable mortality. Overall, this study is not considered sufficiently robust for use in the risk characterisation.

In another study by Chikae et al. (2004b), the effects of DEHP on the fry stage of medaka were studied. The fry were exposed in a semi-static system to the nominal concentrations 0, 0.01, 0.1, 1 and 10 µg DEHP/l (n = 20/group) for three weeks after hatching, starting on day one post-hatch. Ethanol was used as solvent. After three weeks of exposure, fish were transferred to a balanced salt solution for growth. After five months, mortality, body weight, sex ratio and gonadosomatic index (GSI) were measured in the adult fish. The result showed significantly lower body weights in males at 0.01 and 10 µg DEHP/l and in females at 0.1, 1 and 10 µg DEHP/l, although not dose-dependent. A significantly decreased GSI, however not dose-dependent, was observed in males only, and at the concentrations 0.01, 1 and 10 µg DEHP/l. An increase in mortality was observed at 0.1 µg/l and higher, although not significant. No effects on sex ratios could be seen. The results of this study do not demonstrate any dose-related effects. The effects still indicated, i.e. significantly lower GSI, are in conflict with the results in the previous study conducted by the same authors using identical concentrations and species, although at different developmental stages.

The effect of DEHP on vitellogenin production and oocyte development in Japanese medaka was studied by Kim et al (2002). In an acute static exposure test, adult medakas (n =10/group, males and females) were exposed to the nominal concentrations 0, 10, 50 or 100 µg DEHP/l for 5 days. Acetone was used as solvent. At the end of exposure, blood samples were taken and semi-quantitatively analysed for vitellogenin by SDS-PAGE. In a chronic exposure test, fry of medaka (n = 30/group) were exposed semi-statically from 1 or 2 days post-hatch (dph) until the age of 3 months to the nominal concentrations 1, 10 or 50 µg DEHP/l. After three months of exposure, one male and one female from each treatment were sampled and analyzed semi-quantitatively for vitellogenin by SDS-PAGE. For the remaining fish, GSI was determined and histological analysis of the reproductive organs was performed. A test was also performed in order to determine the stability of DEHP in water. An aquaria without fish was diluted to a nominal conc of 50µg DEHP/l, and was analysed for DEHP at 0, 24, 48 and 72 hours.

The results from the water analysis showed that the initial concentration was 88% of the nominal concentration and declined to 57% of the nominal concentration after 72 hours. Time weighted average was 73% of the nominal over 72 hours. In the acute exposure test, no effects on male vitellogenin could be observed, but 2 out of 5 females in the 10 µg DEHP/l group seemed to have lower levels of vitellogenin. In the chronic exposure test females displayed lower levels of

vitellogenin in the DEHP treated groups (one fish/treatment). No induction of vitellogenin was observed in males at any of the test concentrations (one fish/treatment). In males, the results of the GSI analysis showed no effects and no histological effects were seen on testes in any group exposed to DEHP. Females exposed to 10 and 50 µg DEHP/l had statistically significantly lower GSI compared to control fish. Further, retardation of oocyte development was observed in DEHP- treated females. All ovaries of control medaka reached stage II (pre-vitellogenic) or III (vitellogenic) out of three stages, whereas a large proportion of the ovaries from DEHP exposed fish did not. No statistically significant effects on mortality, weight and length was observed in males or femals after 3 months of exposure. The vitellogenin data are weakened by the fact that only one fish per sex has been studied per group. In addition, the relevance of effects on vitellogenin in females can be questioned as it normally used as a biomarker for estrogenic exposure in males. Although a significantly lower GSI could be observed in females, this is not supported by other studies in medaka where, in contrast, effects only have been indicated in males (Chikae et al (2004b). The reporting of the study is poor, affecting the reliability of the study.

In a study by Metcalfe et al (2001), estrogenic properties of DEHP were assessed *in vivo*, in japanese medaka, and *in vitro* in a yeast estrogen screening (YES) assay. In the in vivo study, fry of medaka were exposed from one day post-hatch (dph) until approximately 90 dph in a semistatic system to the nominal concentrations 0, 500, 1,000 and 5,000 µg DEHP/l (n = 60-90/group). Acetone was used as solvent. After sampling, sex ratio, presence of intersex as well as morphometric parameters were studied. In the YES assay, concentrations of DEHP ranging between 50 µg/l – 100 mg/l were tested. No effects on sex ratios, incidence of intersex or morphometric parametres could be observed in the medaka and no estrogenic activity could be detected in the YES assay. The study was considered reliable. However, concentrations above the water solubility of DEHP were used for all groups, indicating that they could have been exposed to the same concentration, i.e. the water solubility limit of DEHP.

Shioda and Wakabashi (2000) studied the reproductive effects of DEHP in a semistatic test on male medakas. Adult males were exposed to the nominal concentrations 39, 120 and 390 µg DEHP/l (n = 3/group) for two weeks. Acetone was used as solvent (< 100 µg/l). Each male were then transferred to dechlorinated tap water and allowed to spawn with two females for two weeks. The number of eggs as well as the hatching rate was examined. No effects of DEHP on the number of eggs or hatchings could be observed. This study is considered to be of poor quality and unreliable.

Exposure via the diet

A few long-term studies where fish have been exposed to DEHP via the diet have also been performed. One of them is a reproduction study while in the other studies the aim has been to study the effect of DEHP on biochemical parameters. These studies are summarised in **Table 3.74**.

In a reproduction study Mayer and Sanders (1973) exposed zebra fish (*Brachydanio rerio*) and guppy (*Poecilia reticulata*) for DEHP via the diet for 90 days. Zebra fish were fed diets containing 50 and 100 µg DEHP/g food, while guppies were fed 100 µg/g food. The number of spawns was greater in DEHP exposed zebra fish compared to control. However, the number of eggs per spawn was reduced and fry survival was significantly ($p < 0.05$) reduced at both exposure levels. For guppies a decrease in fry per female was noted and an 8% incidence of abortions compared to 0% in the control. None of these effects were statistically significant. The DEHP exposed zebra fish fry died in tetany as opposed to the dying control fry were tetany did not occur. Based on the findings that DEHP injected intraperitoneally in Coho salmon

(*Onchorhynchus kisutch*) at 3 µg DEHP/kg fish increased serum calcium (Grant and Mehrle, 1970) the authors suggest that the tetany observed in zebra fish may be an effect of DEHP altering the normal calcium metabolism in fish. This study indicates effects on fry survival. However, due to a high mortality in the control group (49%) this study is not considered valid.

Atlantic cod (*Gadus morhua*) were maintained on a herring diet containing gelatine encapsulated DEHP at 0, 10, 100 and 1,000 µg/g food for 121 days (Freeman et al. 1981). Samples from gonads, head kidneys and livers were incubated with equimolar amounts of [^{3}H]-pregnenolone and [^{14}C]-progesterone for 19 hours. Tissue from male and female fish was treated separately. Extracts of the incubated tissues were analysed with various techniques. There were no significant differences in steroid metabolic profiles in male fish kidneys and testicles at highest dose (1,000 µg DEHP/g food) compared to control. In female fish there was a significant alteration of steroid biosynthetic pathways in the head kidneys and ovaries of the DEHP-fed fish. The isotope ratios of 11-deoxycortisol from 100 and 1,000 µg DEHP/g groups were greater than twice the observed ratios obtained from the control and 10 µg DEHP/g. The significance of this finding is not known.

In order to study the effects of DEHP on lipid metabolism rainbow trout were fed a zoo plankton diet with or without 2% DEHP for seven weeks (Henderson and Sargent, 1983). Liver, adipose tissue and muscle were assayed for lipid content and lipid class composition. The fatty acid composition of the total lipids of these tissues was also analysed. Furthermore, the effects of DEHP on activities of liver enzymes were assayed. There was no significant difference in body weights or liver weights between DEHP fed trout and control fish. The total lipid content in liver of DEHP fed trout was significantly lower ($P < 0.02$) than the lipid content of control fish liver ($3.3 \pm 0.7\%$ and $4.5 \pm 0.2\%$, respectively). Also in adipose tissue the total lipid content was significantly lower ($P < 0.02$) in DEHP fed trout compared to control fish, $41.2 \pm 0.7\%$ and $49.1 \pm 4.7\%$, respectively. The lipid content in muscle did not differ significantly between DEHP exposed fish and control. The lipid class composition in these tissues was similar in DEHP fed fish and control fish. There was also a slight difference in fatty acid composition between DEHP fed fish and control in liver, adipose tissue and muscle. The inclusion of 2% DEHP in the diet had no effect on the enzyme activities assayed for in this study. The relevance of these slight effects on lipid metabolism is not known.

Norrgren et al. 1999, studied the effects on sexual differentiation in Atlantic salmon (S*almo salar*) exposed to DEHP, nonylphenolethoxilate (NPEO) or 17ß-estradiol via the food. Experimental diets were prepared by mixing ethanol solutions of the test compounds with granulated start feed. This resulted in a low and a high dose of 300 and 1,500 mg/kg food respectively for both DEHP and NPEO while the low and the high dose was 15 and 30 mg/kg respectively, for 17ß-estradiol. The control fish received food treated with ethanol only. The ethanol was evaporated from the food before administration to the fish. Each experimental group consisted of 200 individuals kept in separate trough ($1m^3$) supplied with $10\ l\ min^{-1}$ filtered river water. Feeding started in June immediately after yolk sac resorption (approximately 4 weeks after hatch). The fish were fed approximately 2% of their body weight daily for 4 weeks. After that, feeding continued with non-contaminated food for 4 months. At termination of the experiment between 82 and 184 individuals were sampled from each group. The gonads were examined by light microscopy and the liver was dissected and weighed and the liver somatic index (LSI) was calculated. The control group consisted of 49% females while the fish fed diets containing15 and 30 mg/kg 17ß-estradiol developed into 88 and 100% phenotypic females, respectively. The number of females in the groups fed DEHP contaminated food was similar to the control in the low dose (47% females) while it was significantly higher, 64%, in the high dose ($P < 0.05$ chi-square test). The sex ratio in the NPEO fed fish did not differ statistically

from the control. The liver somatic index (LSI) of fish in the high dose was significantly higher (**P < 0.01 ANOVA) than the LSI of control fish, 2.22 compared to 1.74. At the low dose the LSI (1.90) did not differ statistically from the control. Consequently the NOEC in this study was 300 mg/kg food and the LOEC 1,500 mg/kg food both for sex ratio and LSI.

In addition to the feeding trial juvenile salmon weighing approximately 7.5 g were injected intraperitoneally with different test compounds in order to study induction of vitellogenin synthesis. Each group consisted of 5 fish that were kept in separate aquaria (50 l). The group injected with DEHP received a total dose of 160 mg kg^{-1} bw during 17 days. At termination of the exposure period blood was sampled from the fish and analysed for vitellogenin. No vitellogenin was detected in the blood of the DEHP injected fish.

In a follow up study to the Norrgren study (Norman et al., 2007), Atlantic salmon were fed with DEHP contaminated food with nominal concentrations of 0, 400, 800 and 1,500 mg/kg food (dwt). The mean measured concentrations, based on measurements at the start and the end of the exposure period, was 358, 827 and 1,648 mg/kg for the three exposure levels, respectively. Feeding with DEHP contaminated food was initiated at the end of the yolk sac stage and continued for 4 weeks as in the first study. Hereafter the fish were fed uncontaminated food. Each exposure group consisted of approximately 1,000 individuals. Sampling was performed after 4 and 9 month. In this study no effects on the sex ratio were observed. However, a 6% incidence of ovotestis in males, which was statistically significant compared to the control were no ovotestis was observed, occurred in the highest dose group (1,500 mg/kg dwt) after 4 month. After 9 month an incidence of ovotestis of 1% (not statistically significant) was observed. Also at 800 mg/kg ovotestis was observed both after 4 month and 9 months, however not statistically different from the control group. The findings indicate that the effects obtained are reversible as no significant effects were seen after 9 month but only after 4 month. It can be concluded that the LOEC from this study is 1,500 mg DEHP/kg and the NOEC is 800 mg DEHP/kg. There is no obvious explanation to the stronger response at the highest dose level (significantly changed sex ratio) seen in the study by Norrgren et al. compared to the effects at the highest dose level in this study (low incidence of ovotestis). However, as no analytical confirmation of the exposure concentrations was made in the earlier study the authors of the present study speculates that the stronger response seen in the Norrgren study may have been caused by a higher exposure than intended. Based on the results from both studies it is concluded that the NOEC for effects on sexual differentiation of Atlantic salmon is 800 mg/kg food.

In addition to the histopathological analyses DEHP and MEHP concentrations in the exposed fish were measured. At the termination of the uptake period the concentrations of DEHP was 0.66, 1.3 and 2.6 mg/kg wet weight in the three exposure levels respectively. The correspondeing MEHP concentrations were 0.22, 0.50 and 0.76 mg/kg wet weight. Based on this and the measured exposure concentrations lipid normalised BMF factors were calculated. The BMF factors for DEHP was 0.011, 0.0064, 0.0073 for the three exposure levels, respectively.

To further elucidate possible effects on fish from DEHP during long term exposure Brixham Environmental Laboratory, Astra Zeneca, UK has performed a multi-generation study with fathead minnow (*Pimephales promelas*) sponsored by the European Council for plasticizers and Intermediates (ECPI) (Caunter et al., 2004). The study followed a protocol based on a guideline adapted from USA EPA Fish Life-Cycle Toxicity test; EPA 540/9-86-137, July 1986, incorporating biological endpoints (vitellogenin and gonad histopathology).

The study comprised three generations, F0, F1 and, F2 of Fathead minnow (*Pimephales promelas*) with a total length of exposure of 472 days. The fish were exposed to DEHP both via water and via the food. Two treatments, Low Dose Food (LDF) and High Dose Food (HDF)

were employed, both having the same nominal DEHP concentration in the water (5.0µg/l) but differing with respect to the concentration in the food, 125 mg/k and 500 mg/kg, respectively The mean measured DEHP concentration water was 3.2µg/l in the the LDF-group and 2.6µg/l in the HDF-group. The mean measured DEHP concentration in the food was 115 mg/kg in the LDF-treatment and 474 mg/kg in the HDF treatment. In addition to this a dilution water control (DWC) and a solvent control (SC) were employed. (see **Table 3.60**). During early development, from hatch up to day 15 post hatch the fry were fed on live food, freshwater rotifers and Artemia. This food was not "amended" with DEHP (DEHP exposure via the water only). From day 16 post hatch for each generation, fish were given DEHP amended food. Each treatment and control was run in duplicate.

Table 3.60 Nominal and measured concentrations of DEHP in food and water of exposure groups and controls

Treatment groups	Nominal conc. of DEHP in the water (µg/l)	Mean measured conc. of DEHP in the water (µg/l)	Nominal conc. of DEHP in the food (mg/kg)	Mean measured conc. of DEHP in the food (mg/kg)
Dilution Water Control (DWC)	-	< 0.25	-	< 0.25
Solvent control (SC)	-	< 0.25	-	< 0.25
5.0 low dose food (LDF)	5.0	3.2	125	115
5.0 high dose food (HDF)	5.0	2.6	500	474

DEHP deuterated in the aromatic ring with a purity of 99.3% was used in the study. The stock solution used for the exposure via water was prepared by dissolving DEHP in tri ethylene glycol (trigol). The concentration of trigol in the treatment groups and in the solvent control was 2.5µl/l. DEHP dissolved in acetone was used to prepare the fish food. The control food was treated with acetone only.

The fish were exposed in a dynamic flow through system with all equipment constructed of glass. The flow was 7 tank volumes/day in the tanks used for adult fish and 12 tank volumes per day in the progeny tanks. The nominal temp during the study was 25±1^{o}C with a photoperiod of 16 hours light: 8 hours dark and a 20 minutes dawn/dusk transition period.

The test procedure will not be described in detail in this summary but is briefly summarised in **Table 3.61**.

The endpoints studied were the following:

F0

- Hatching success
- Length and weight 100 dph (days post hatch)
- Egg production

In addition to this samples for VTG (vitellogenin) and histopathology were taken but not analysed.

F1

- Hatching success
- Survival 28 dph

- Length and weight (28 and 100 dph)
- Egg production

In addition to this samples for VTG and histopathology were taken but not analysed.

F2

- Hatching success
- Survival 28, 42 and 100 dph
- Length and weight (28 and 100 dph)
- VTG conc males and females
- Gonad hisopathology (sex ratio)

In addition to this 11-12 fish (5-6 per replicate) in the LDF and HDF treatments from the F0 and F1 generations were sacrificed when approximately 200 days old and analysed for DEHP and MEHP.

Table 3.61 Overview of the test procedure

Exposure day (date)	Post-hatch day	Post-hatch day	Study		Number of				In life measurements	
FO	***F1***	**F2**	**Phase**	**Details**	**Reps**	**Tanks per rep**	**Animals per tank**	**Animals per treatment**	**Length**	**Weight**
			Embryos <24 h old	Dead embryos discarded between start and hatching	2	4*	25	200		
4 (24.11.2001)			Hatch day (embryos to larvae)	Larvae released into progeny tanks, numbers of larvae reduced, excess larvae sacrificed and discarded	2	2	35	140		
45 (04.01.2002)			Larvae transferred to adult tanks	Growth of fish requires transfer to larger tanks Excess fish deep frozen at - 18°C	2	1	50	100		
104 (04.03.2002)			Sample maximum of 25 fish per tank	Fish sampled for possible vitellogenin and histopathology	2	1	25	50	x	x
111 (107 dph) (11.03.2002)			25 adults used to create 4 breeding pairs (4x2=8 adult fish per tank) Spare adults (max. 17) held in progeny tanks	Fish reach sexual maturity and breeding pairs created. After pairing fish, spawning tiles are introduced into breeding chambers; reproduction starts soon thereafter	2	1	8	16		
121 (21.03.2002)			Spare adults sacrificed	Spare fish deep frozen at - 18°C	2	2	4/5	9	x	x
125-194 122-141 169- 189	*0* *0*		*F1 hatchabilities (2 per breeding pair)* *Begin 1st F1 ELS tests (FE1)* *Begin 2nd F1 ELS test (F1P)*	Measure % embryo hatch *Continues to become Fl generation*						
154- 173	*28*		*1st F1 ELS tests end after 28 days post hatch*	*Fish sampled for weight and length*	2	1	Max 50	Max 100	x	x
204 (12.06.2002)			End of FO	Remaining F0 adults (max 8) sacrificed fish deep frozen at- I8°C	2	2	4	8	x	x
273-293	*100*		*Sample maximum of 30 fish per tank*	*Fish sampled for possible vitellogenin and histopathology*	2	1	30	60	x	x

Table 3.61 continued overleaf

Table 3.61 continued Overview of the test procedure

Exposure day (date)	Post-hatch day	Post-hatch day	Study		Number of				In life measurements	
FO	***F1***	**F2**	**Phase**	**Details**	**Reps**	**Tanks per rep**	**Animals per tank**	**Animals per treatment**	**Length**	**Weight**
	107		*25 adults used to create 4 breeding pairs* (4x2=8 *adult fish per tank)* *Spare adults (max. 17) held in progeny tanks*	*Fish reach sexual maturity and breeding pairs created. After pairing fish, spawning tiles are introduced into breeding chambers; reproduction starts soon thereafter*	2	1	8	16		
302-375 300-326 349-367		0 0	F2 hatchabilities (2 per breeding pair) Begin first F2 ELS tests F2E1) Begin second F2 ELS tests F2E2)	Measure % embryo hatch						
384-393 Ends 18.12.02	*Approx* 200		*End of F1*	*Remaining F1 adults (max 8) sacrificed fish deep frozen at-18°C*	2	2	4	8	x	x
		28	F2E1 tests end after 28 days post	Fish sampled for weight and length	2	1	Max 50	Max 100	x	x
		100	F2 E2 tests end after 100 days post hatch	Fish sampled for possible vitellogenin and histopathology	2	1	Max 30	Max 60	x	x
453-472 Ends 07.03.03		100	End of F2	Remaining adults (max 8) sacrificed fish deep frozen at-18°C	2	2	4	8	x	x

* Incubation cups are sub units (2 incubation cups per progeny tank, 2 progeny tanks per replicate)

The results from the study are presented in **Table 3.62** - **Table 3.68**.

Data was tested for normality and depending on the outcome, differences between treatments and control were statistically analysed with parametric- (ANOVA, t-test) or non parametric-tests (Wilcoxon´s Rank Sum Test). The survival was statistically analysed using a contingency table procedure. If there were statistically significant differences between dilution water and solvent control the solvent control (SC) was used for the statistical analysis, if not pooled controls was used.

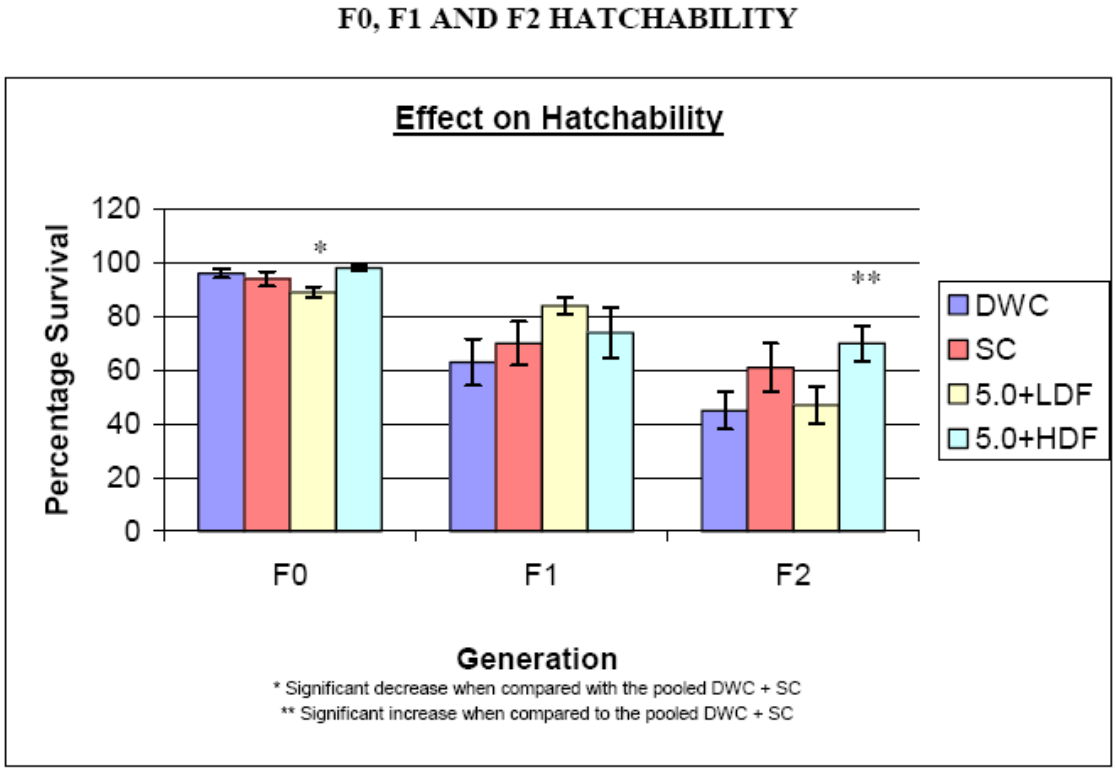
The hatchability was significantly lower in the LDF (low dose fish) treatment for the F0 generation whereas no difference was observed in the HDF (high dose fish) treatment or in any of the treatments of the F1 and F2 generations (see **Table 3.62** and **Figure 3.5**). Overall the hatchability decreased for each generation. However, as the decrease was of a similar magnitude both in the DEHP treated groups and the controls it is probably not related to the exposure to DEHP.

Table 3.62 Effects on hatchability

Egg hatch (%)	Treatment			
	5.0 + LDF	5.0 + HDF	SC	DWC
F0 (mean ± SD)	89 ± 4.2[a]	98 ± 1.9	94 ± 5.3	96 ± 3.3
F1 (mean ± SD)	84 ± 12	74 ± 32	71 ± 30	63 ± 33
F2 (mean ± SD)	47 ± 28	70 ± 29	61 ± 35	45 ± 29

a) Significant reduction (P=0.05) in egg hatch compared with the pooled controls

Figure 3.5 Effects on hatchability (graph taken from Caunters et al., 2004)



The survival data were analysed by contingency table tests. The survival in the F1 generation was similar to the pooled controls in the LDF treatment but significantly lower in the HDF treatment. In the F2 generation the survival was significantly lower in the LDF treatment whereas it was unaffected in the HDF treatment (see **Table 3.63**). The results indicate effects of the DEHP exposure. However, due to the inconsistencies in the results (lack of dose response in the F2 generation) it is not possible draw firm conclusions.

Table 3.63 Effects on survival

Survival (%)		Treatment			
		5.0 + LDF	5.0 + HDF	SC	DWC
F1	28 dph	92	78[d]	96	85
F2	28 dph	71[a]	101	95	83
	42 dph	68[b]	92	93	90
	100 dph	61[c]	92	77	77

a) Significant reduction (P=0.0001) in survival compared with solvent control (SC)
b) Significant reduction (P=0.0001) in survival compared with pooled controls
c) Significant reduction (P=0.01) in survival compared with pooled controls
d) Significant reduction (P=0.0004) in survival compared with solvent control (SC)

For the growth endpoints weight and length a number of statistically significant differences were observed (see **Table 3.64**). Due to skewed distributions the statistical analysis was performed with non- parametric tests (Wilcoxon's rank sum test). In most cases the solvent control was used for comparison due to statistically significant differences between the two control groups. In 4 out of 10 treatments, F0: LDF and HDF; F1: LDF 28 dph and F2: LDF, 28 dph the mean length of the fish was significantly different from the controls. In 3 of these 4 cases the DEHP treated fish were longer than the control fish and in one case shorter. Regarding the weight 4 treatment groups had significantly higher body weights compared to the control (F0: HDF, F1: LDF and HDF, 28 dph and F1: LDF, 100 dph) whereas 3 groups had significantly lower bodyweights compared to control (F2: LDF and HDF, 28dph and F2: LDF 100 dph). Due to the large variation and the variable results, sometimes indicating increases and sometimes decreases from the exposure of DEHP in addition to frequent statistical differences between the dilution water control and the solvent control it is not possible to draw any firm conclusions on the possible effects of DEHP on growth from this study.

Table 3.64 Effects on growth

Growth (mean ± SD)		Treatment			
Generation	Endpoint	5.0 + LDF	5.0 + HDF	SC	DWC
F0	Length 100 dph (mm)	37.4[a] ± 4.18	38.4[a] ± 3.27	36.3 ±3.24	35.7 ± 3.54
	Weight 100 dph (g)	1.05 ± 0.46	1.14[a] ± 0.33	0.93 ± 0.29	0.94 ± 0.31
F1	Length 28 dph (mm)	17.2[b] ± 2.14	17.2 ± 1.3	16.9 ± 1.21	17.3 ± 2.01
	Length 100 dph (mm)	40.2 ± 5.48	38.9 ± 3.58	39.1 ± 4.09	37.1 ± 3.51
	Weight 28 dph (g)	0.091[b] ± 0.034	0.082[b] ± 0.020	0.072 ± 0.018	0.094 ± 0.031
	Weight 100 dph (g)	1.17 ± 0.34	1.46[a] ± 0.63	1.28 ± 0.45	1.14 ± 0.35

Table 3.64 continued overleaf

Table 3.64 continued Effects on growth

Growth (mean ± SD)		Treatment			
Generation	**Endpoint**	**5.0 + LDF**	**5.0 + HDF**	**SC**	**DWC**
F2	Length 28 dph (mm)	17.8 ± 2.76	18.2 ± 1.52	18.7 ± 2.35	16.5 ± 2.17
	Length 100 dph (mm)	38.6[b] ± 4.33	39.3 ± 4.33	40.5 ± 4.84	36.6 ± 6.75
	Weight 28 dph (g)	0.098[b] ± 0.048	0.099[b] ± 0.026	0.120 ± 0.043	0.078 ± 0.030
	Weight 100 dph (g)	1.38[b] ± 0.54	1.45 ± 0.53	1.60 ± 0.63	1.22 ± 0.65

a) Bold italic numbers indicate statistically significant differences compared to pooled controls
b) Bold numbers indicate statistically significant differences compared to solvent control.

No statistically significant effects on egg production were observed (ANOVA, t-test), neither when based on the mean total number of eggs produced per female nor when based on mean number of eggs produced per female and reproductive day (see **Table 3.65**). It should be noted however, that due to the very large variation in egg production between individual females a much larger material would have been needed to make it possible to reveal any statistical differences between the groups than the 8 females/treatment used in this study.

Table 3.65 Effects on egg production

Egg production	Treatment			
	5.0 + LDF	**5.0 + HDF**	**SC**	**DWC**
F0 (No. of eggs /female)	1,184 ± 751	1,234 ± 765	1,550 ± 1,103	1,528 ± 1,316
(No. of eggs/female/day[a])	13.1 ± 7.7	13.3 ± 8.2	17.0 ± 11.4	16.4 ± 14.2
F2 (No. of eggs /female)	1,449 ± 1,260	1,763 ± 1,292	1,865 ± 1,452	1,315 ± 985
(No. of eggs/female/day[a])	15.7 ± 13.4	19.8 ± 13.4	20.5 ± 15.9	14.2 ± 10.6

a) Reproductive day

For the F2 generation histopathology and vitellogenin analysis was performed.

The vitellogenin concentrations were highly variable and almost twice as high for the males in the HDF treatment compared to the pooled controls (see **Table 3.66** and **Figure 3.6**). The distributions were not normal and the difference was not statistically significant when analysed with Wilcoxon´s rank sum test. For the females a statistically significant increase in vitellogenin concentrations of approximately 50% compared to the pooled controls was observed using parametric statistical tests (ANOVA, t-test). A statistical analysis performed by the rapporteur shows that the exposed group was normally distributed but the control group had a skewed distribution and therefore a non-parametric test should have been used. However, also non-parametric statistical methods (Mann-Whitney u-test) results in a statistically significant difference between the exposed group and the pooled controls. When compared to the solvent control no significant increase was observed. The authors of the study argues that the solvent control is the more relevant to use because evidence of effects of the solvent itself is often seen in VTG studies. In this study however, there was no statistical difference between the two control groups, which means that the comparison should be made using the pooled controls and the conclusion must be that there is a statistically significant increase in vitellogenin concentration in the HDF females.

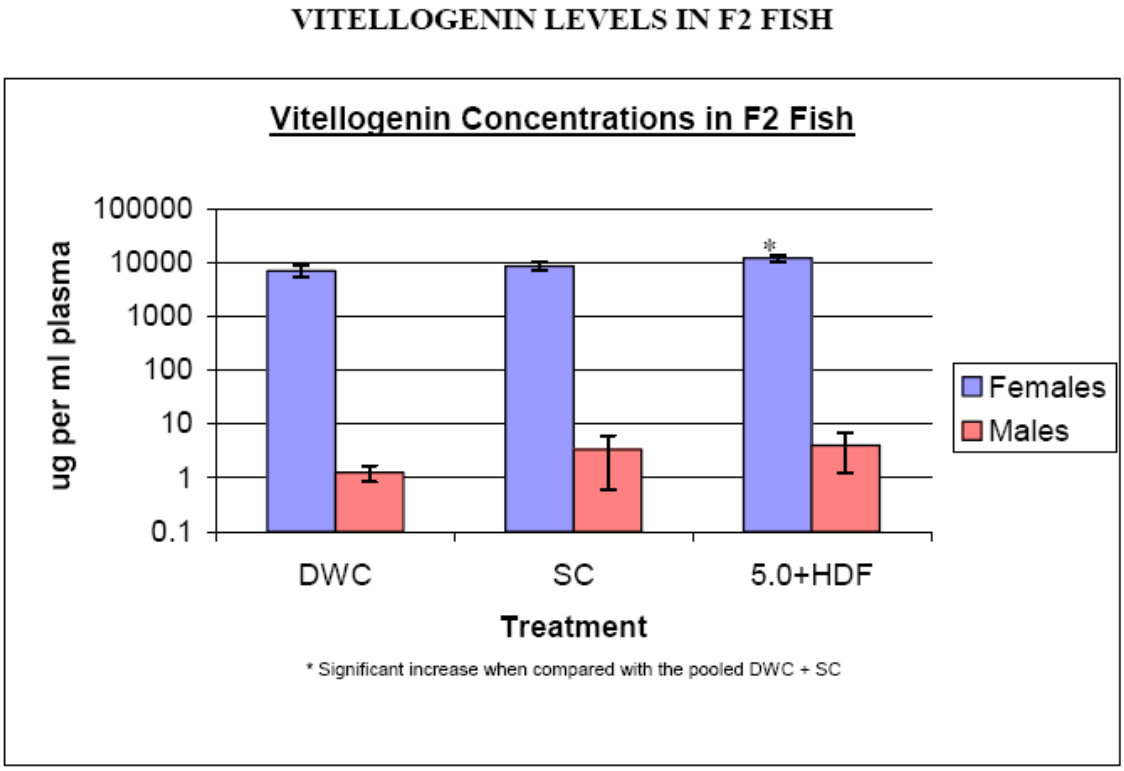
However, due to the large variation and the small material this may as well be a random finding. Furthermore, the ecological relevance of a small increase in female vitellogenin levels is questionable. In this respect it is a big disadvantage that VTG has not been analysed also from the F2 low dose group as well as from the F0 and F1 generations.

Table 3.66 Effects of DEHP on vitellogenin concentrations of theF2 generation.

Vitellogenin conc. (ng/ml)	Treatment			
	5.0 +LDF	5.0 + HDF	SC	DWC
Males (arithmetic mean ± SD)	-	4,032 ± 13,231	3,384 ± 11,429	1,258 ± 2,038
Geometric mean		658	415	469
Females (arithmetic mean ± SD)	-	12,043,590[a] ± 7,420,191	8,600,251 ± 7,346,614	7,030,970 ± 6,655,941
Geometric mean		8,019,293	4,634,329	3,872,999

a) Statistically significant difference compared to pooled controls

Figure 3.6 Vitellogin concentrations in F2 fish (graph taken from Caunters et al., 2004)



No effect of DEHP exposure on the sex ratio was observed (see **Table 3.67**). The large variation especially between the replicates in the DEHP treated group makes these results hard to interpret and statistical analysis not meaningful. To be able to draw a firm conclusion the material should have been larger. An analysis of the Low dose group as well as the F0 and F1 generations would possibly have given more reliable results.

Table 3.67 Effects on sex ratio (F2 generation)

Treatment	Replicate	Males		Females		Intersex
		(N)	(%)	(N)	(%)	(%)
5.0 + HDF	A	4	55	16	45	0
	B	18		2		
SC	A	7	40	13	58	3
	B	9		10		
DWC	A	12	62	5	39	0
	B	12		11		

The gonadal development was also studied. The results are presented in **Table 3.68**. For females there were no obvious differences between the HDF group and the control. For males however, the proportion individuals with immature gonads was lower than in the controls whereas the proportion of fish with moderate spermatogenesis was higher. However, also for these findings the variation between replicates was large which meant that no meaningful statistical analysis could be performed.

Table 3.68 Effects on gonadal development based on total of individual numbers in each treatment

		Treatment		
Gonadal development		**5.0 + HDF**	**SC**	**DWC**
Male (N)		**(22)**	**(16)**	**(24)**
Immature	(%)	4.5	25	25
Minimal spermatogenesis	(%)	4.5	37.5	25
Slight spermatogenesis	(%)	23	6	12.5
Moderate spermatogenesis	(%)	68	31	37.5
Female (N)		(18)	(23)	(16)
Previtellogenic follicles only	(%)	6	4	19
Minimal vitellogenic follicles	(%)	6	9	0
Slight vitellogenic follicles	(%)	11	9	6
Moderate vitellogenic follicles	(%)	78	78	75

In summary, a number of statistically significant differences between treatments and controls were observed in the study. In most cases however, these differences were probably not caused by the exposure of DEHP. Due to the large variation, frequent statistically significant differences between dilution water and solvent control groups, inconsistent and often not dose related responses in the DEHP treated groups and for some endpoints a small group size, it is hard to draw firm conclusions regarding the effects seen. However, the exposure levels in the study were fairly high, much higher than normally seen in the environment. And since no serious effects that can be unequivocally attributed to the exposure of DEHP were observed, the study gives a fair indication that serious effects probably should not be anticipated at more environmentally realistic exposure levels.

The bioaccumulation part of the study is reported in an addendum to the main report.

The wet weight concentration of DEHP in F0 fish from the LDF and HDF groups was fairly similar 2,204 µg/kg and 2,040 µg/kg, respectively. However, in the F1 generation the DEHP concentration in the HDF group was approximately twice that of the low dose group, 646 µg/kg and 1,396 µg/kg, respectively (see **Table 3.69**). The MEHP concentrations were more than an order of magnitude lower than the DEHP concentrations. Calculated Bioconcentration factors for DEHP based on the assumption that the uptake was from the water only ranged between 202 and 785 for the different groups. When including MEHP the BCF:s ranged from 217 to 825.

Table 3.69 Concentrations of DEHP and MEHP and corresponding BCF- values

Treatment	N	Mean DEHP concentration (ug/kg wet fish)	Mean MEHP concentration (ug/kg wet fish)	Mean measured DEHP concentration in water (ug/l)	BCF[a]	BCF[b]
F0: 5.0 + LDF	11	2,204	79	3.2	689	713
F0: 5.0 + HDF	12	2,040	106	2.6	785	825
F1: 5.0 + LDF	12	646	48	3.2	202	217
F1: 5.0 + HDF	11	1,396	105	2.6	537	577
Mean BCF					**553**	

a) Based concentration of DEHP in fish
b) Based on concentration of DEHP + MEHP in fish

Calculating BMF factors with the assumption that all uptake was from the food gave BMF factors ranging between 0.01 and 0.06 for the different groups (see **Table 3.70**). When lipid normalised the BMF factors ranged from 0.01 to 0.07.

Table 3.70 Biomagnification factors

Fish	Mean DEHP[a] (mg/kg dry fish)	Nominal DEHP in food (mg/kg dry weight)	BMF	Mean DEHP[1] (mg/kg fish lipid)	DEHP in food[2] (mg/kg lipid)	BMF (lipid normalised)
F0:LDF	7.71	125	0.06	58	781	0.07
F0:HDF	7.13	500	0.01	54	3,125	0.02
F1:LDF	2.26	125	0.02	17	781	0.02
F1:HDF	4.88	500	0.01	37	3,125	0.01
Mean BMF			**0.03**			**0.03**

a) Using mean dry weight = 28.6% of wet
b) Using lipid concentration in wet fish = 3.8%
c) Using lipid concentration in dry food = 16%

It is not possible from this study to separately quantify the uptake from the two exposure routes or even to draw any firm conclusions regarding the relative importance of the two exposure routes. At least for the F0 fish the mean measured DEHP concentrations in fish do not reflect the 4:1 ratio of the food level between the HDF (500 mg/kg in food) and LDF (125 mg/kg in food) indicating that the main route of uptake in this study is via the water. This is also supported by the calculated BCF factors which are rather similar to many of the literature BCF values for fish. On the other hand, the DEHP concentrations in F1 fish are more than twice as high in the HDF group compared to the LDF group, despite a slightly lower exposure via the water. This is an indication that the exposure via food in this study may be as important as the exposure from water.

Summary of fish toxicity

From the effect studies on fish it can be concluded that DEHP have no acute effects at exposure levels far exceeding its apparent water solubility.

No significant mortality was seen in the long-term toxicity studies with juvenile and adult fish. However, there are indications that DEHP may have effects on growth at relatively high exposure concentrations. Defoe et al. (1990) noted a statistically significant weight reduction of 13% when juvenile Japanese medakas were exposed to a mean measured concentration of 0.554 mg DEHP/l for 168 days. When fertilised rainbow trout eggs and resulting fry were exposed to DEHP for a total of 90 days a weight reduction of approximately 10% was observed at 0.259 and 0.502 mg/l which were the two highest exposure levels. These weight reductions were not statistically significant (Defoe et al., 1990) but may be an indication of effects on growth. The slightly impaired growth in these studies may be an effect of physical influence of the test substance as the test concentrations were well above the "true" water solubility. On the other hand the effects on growth may be a result of DEHP affecting the collagen synthesis in fish. Mayer et al. (1977) observed effects on collagen synthesis at exposure levels as low as 0.004 mg/l when three different fish species were exposed to DEHP at concentrations up to 0.1mg/l. However, no effects on growth were seen in this study and the biological and ecological significance of the effects on collagen synthesis is unknown. Therefore, these results are not considered relevant to use in the risk assessment.

In the embryo larval studies effects were indicated at lower exposure levels and the most sensitive life stage seems to be the period between hatch and swim up (yolk adsorption). The lowest NOEC is 0.005 mg/l for rainbow trout (Mehrle and Mayer, 1976). However, the results from this study are not considered valid for the purpose of risk assessment as discussed earlier (see Section 3.2.1.1.1). In a semi-static study with channel catfish (Birge et al., 1978) the mortality was 10% at a nominal concentration of 0.1 mg/l. However, it is uncertain if the effects seen are due to the intrinsic toxicity of DEHP as effects were seen only at nominal concentrations well above the true water solubility for fresh water. Another reservation for this study is that the carrier solvent concentration differed between the different DEHP exposure levels while the solvent concentration of the control was not stated.

The available studies on Japanese medaka gave very varying and contradictory results. It shall be noted, though, that the studies are not directly comparable with each other since they are different with respect to exposure periods, endpoints and exposure concentrations. In two of the studies no effects of DEHP exposure was seen (Metcalfe et al., 2001, Shioda and Wakabashi, 2000). In the other three studies (Chikae 2004 a nd b, Kim 2002) various effects were observed indicating both estrogenic or antiandrogenic as well as antiestrogenic effects. Most of the effects observed were not dose-dependent. Furthermore, the quality of some of the studies is questionable and the results from these studies are considered not useful for the quantitative risk assessment.

No firm conclusions can be drawn from the few studies where the effects of DEHP on biochemical parameters have been studied. Effects on collagen and hydroxyproline synthesis have been demonstrated. The dose-response relationship was lacking or weak and no effects on growth were seen. Furthermore, it seems like DEHP has slight effects on lipid metabolism and steroid synthesis when administered via the feed at relatively high concentrations. The biological significance of these findings is uncertain.

In conclusion, there is no reliable long-term study indicating effects below the "apparent" water solubility of DEHP. Therefore, it is not considered suitable to specify a chronic NOEC for fish exposed via water.

In a three generation study on fathead minnow (Caunters et al., 2004), the effects on a large number of endpoints of simultaneous exposure via food and water was studied. A number of statistically significant differences between treatments and controls were observed in the study. However, due to the large variation, frequent statistically significant differences between dilution water and solvent control groups, inconsistent and often not dose related responses in the DEHP treated groups and for some endpoints a small group size, it is hard to draw firm conclusions regarding the effects seen. In most cases they were probably not caused by the exposure of DEHP. It is therefore, not possible to derive a NOEC from the study and thus it is not possible to use the results from the study in a quantitative risk characterisation. The exposure levels in the study were fairly high, much higher than normally seen in the environment. Since no serious effects that can be unequivocally attributed to the exposure of DEHP were seen the study indicates that serious effects should probably not be anticipated at environmentally more realistic exposure levels.

However, there are studies showing effects of DEHP when fish are exposed via the food. For instance, there are indications of increased fry mortality from a study where zebra fish were fed DEHP in the diet for 90 days (Mayer and Sanders, 1973). This study is not considered valid, in order to obtain a NOEC for the risk assessment, because the mortality in the control was too high.

In a study by Norrgren et al. (1999) effects on the sex ratio of Atlantic salmon was observed when the fish were exposed to a nominal concentration of 1,500 mg DEHP/kg food. In a follow up study (Norman et al., 2007) a weaker response was observed. There were no effects on sex ratio, but a statistically significant increased incidence of ovotestis in the highest dose group (1,500 mg/kg food), whereas the slight increase seen at 800 mg/kg was not statistically significant. Thus the NOEC from this study was 800 mgDEHP/kg food.

The commercial food used in these two studies is a very high quality food. The approximate food conversion from this diet is; ingestion of 1 g of food results in 1 g of increase in body weight. The corresponding food conversion from natural diets is: 1 g food gives 0.2 g increase in body-weight (L. Norrgren, pers. comm.). This difference is in part due to the difference in water content between dry pelleted food and natural food. To take account of this the NOEC of 800 mg/kg is recalculated to wet weight basis using a factor of 5. The NOEC for natural diets then becomes 160 mg DEHP/kg food (wwt). This NOEC will be used in the risk assessment.

3.2.1.2 Toxicity to amphibians

Some frogs (e.g. *Rana arvalis*) spend part of their lives, as eggs and newly hatched tadpoles, on the sediment surface. Therefore frogs have been used in toxicity studies with toxicants in water as well as in sediment. Effects on hatching and survival of tadpoles may be regarded as indications of sediment toxicity. Burton (1992) reviewed the literature on sediment toxicity studies with amphibians. One conclusion was that the duration of embryo contact with the sediment was an important factor mediating exposure. According to a SETAC guidance document on sediment toxicity tests (Hill et al., 1994), amphibian embryo-tests reasonably fulfil the criteria for sediment tests.

In studies on amphibians exposed to DEHP, some involve exposure via water only and some exposure of the amphibians to spiked sediments. The toxicity data for DEHP on amphibians are summarised in **Table 3.75**.

Exposure via water

Birge et al. (1978) exposed one frog-species (*Rana pipiens*) and one toad-species (*Bufo fowleri*) to DOP (actually DEHP according to the Canadian Water Criteria Guidelines (1993)) at nominal concentrations between 0.01 and 100 mg/l. The organisms were exposed for 7-8 days, from newly fertilised eggs until 4 day old tadpoles. Since the tests cover sensitive life stages they are considered to be long-term. Different volumes of acetone were added depending on toxicant concentration. It is not clear if each test concentration was compared to a control with similar solvent volume. The reported LC_{50} values were 4.44 and 3.88 mg/l for the two species respectively. NOEC values were not mentioned. (In the test with *B. fowleri*, the tadpoles were ten times more sensitive to the test substance, compared to the eggs. This was not the case with *R. pipiens*.) The most sensitive test organisms, tadpoles of *B. fowleri,* showed 5% mortality at 0.1 mg DEHP/L and 25% mortality at 1 mg/l. Both for *B. fowleri* and for *R. pipiens* a dose-response relation was observed for mortality at DEHP concentrations ≥ 1mg/l. Since only nominal concentrations are reported and since the effects start to occur at or above the 'apparent' water solubility these results cannot be used for the purpose of this risk assessment.

Dumpert and Zietz (1983) investigated the effect of DEHP on the clawed toad *Xenopus laevis* in two long-term studies. In both studies the organisms were exposed from being newly spawned until fully developed frogs. In the first study a solvent, methyl alcohol, was added. Decreased survival, taken as an embryo-toxic effect of DEHP, was indicated but did not occur as a dose-response relationship. Delayed development was observed in systems with DEHP additions as well as in the solvent control. This was taken as a sign of growth inhibition or of teratogenic effects of methyl alcohol. This study is therefore considered invalid for the purpose of this risk assessment.

The same authors performed another experiment, without solvent, exposing the same species to repeated weekly applications of one concentration of DEHP. At 2 mg DEHP /L (measured) the development time (egg to fully developed frog) was doubled compared to the control. The pigmentation of the frog was also affected. This effect was not quantified. Hence, the LOEC from this study is 2 mg/l and the NOEC is < 2 mg/l.

These studies indicate that amphibians might be sensitive to DEHP at high concentrations. But since the reported effect concentrations (of which only one is measured) are above both the 'true' as well as the 'apparent' water solubility, they cannot be used in order to derive a $PNEC_{water}$ for this risk assessment.

Exposure via sediment

Three studies with moor-frog (*Rana arvalis*) eggs exposed to DEHP contaminated sediments have been carried out. The eggs used in all experiments were collected in the field (in Sweden). The static test systems consisted of natural sediment, spiked with DEHP, and overlaying water to which clumps of fertilised eggs were added.

In the first study (Larsson and Thurén, 1987, referred to as Study I in the discussion below) 2-3 day old eggs were exposed to DEHP in sediment at six test concentrations, the nominal values ranging between 10 and 800 mg/kg WWT. The eggs hatched after three weeks and the tadpoles were then exposed for three more weeks. Egg hatching success was determined by

counting the tadpoles after 5, 12 and 30 days. Tadpole survival was determined at the termination of the exposure, after 60 days. Successful hatching decreased with increasing DEHP levels in the sediment while, after hatching, the survival rates of the tadpoles did not differ from that in the reference group. The hatching results are reported as a dose-response curve with standard deviation (SD) for each tested concentration plotted in a diagram. The authors report an EC_{50} of 150 mg/kg (WWT) for the hatching of tadpoles. LOEC and NOEC values are not reported. For the purpose of this assessment, SD was read from the diagram and used in one-tailed T-test in order to evaluate if the reported mean effect percentages significantly differ. LOEC was then determined as the first concentration that significantly ($p < 0.05$) differs from the control. The next lower dose was set as NOEC. This evaluation of the data resulted in a NOEC of 8.8 mg/kg (WWT) (measured). The authors report a dry weight of 25-40% of the fresh sediment. If a mean dry weight of 33% is applied on the NOEC above, this result in a dry weight based NOEC of about 25 mg/kg (dwt).

In the second study by Wennberg et al. (1997), referred to as study II, 4 days old eggs were exposed to DEHP contaminated sediment. The nominal test concentrations ranged between 15 and 600 mg/kg (dwt). Sediments with different organic contents (3 - 77% LOI) were also tested at one test concentration, 300 mg/kg (dwt). The endpoints were embryo hatching (monitored once a week) and tadpole survival and growth, determined at the end of the experiment. The hatching of tadpoles started between 8 and 14 days of exposure and the experiment was terminated after 29 days. No significant effect on hatching or survival of tadpoles was observed in any of the test concentrations. Neither was any effects observed in sediments with differing organic contents (and about 300 mg/kg (dwt) of DEHP). The highest measured test concentration (NOEC) was 433 mg/kg (dwt).

Questions were raised regarding the spiking method and the temperature used in Study I. Therefore the second study was carried out, applying another spiking method and a higher temperature. The two studies differed in the following aspects.

a) Spiking method

In study I, DEHP dissolved in ethanol was mixed directly into wet sediment. The amount of ethanol used is not reported, but the same amount was used in all treatments (P. Larsson, pers. com.). About 200g of the spiked sediments was placed in 2-litre bottles and was left for evaporation overnight before 1 litre of water was added. Each such test system was then "continuously and gently" aerated for one week before the eggs were added. In Study II the spiking method of Brown et al. (1996) was adopted. DEHP was added with acetone (the amount not reported) into dried sediment followed by evaporation of the solvent under reduced pressure, in an evaporator. The spiked, dry, sediment was then blended into wet sediment and equilibrated on a shaking board for one week before the sediment-water systems were arranged and eggs added.

It has been shown, for some poorly soluble organic substances, that the presence of ethanol and acetone can modify the acute toxicity of the compound to aquatic invertebrates. Since the mechanisms for synergism and antagonism for mixtures are poorly understood, the use of solvents should be avoided if possible (ECETOC, 1996). Another limitation for the use of solvents like acetone and ethanol is their ready biodegradability, which might lead to oxygen depletion in the test solution. Elimination of such solvents prior to the start of the test "by application of adequate methods" may reduce such problems (ibid.). However, also if the solvent (in this case acetone) is evaporated, it is likely that the drying of the sediment and the extraction effects of the concentrated acetone markedly affect the adsorption capacity of the sediment.

It is not likely that the observed effects in study one were caused by oxygen depletion, since a dose-response effect curve was observed, and the same amount of ethanol was added in all tests systems. Furthermore, a spiking method control was included in study II; DEHP in 2.75 ml ethanol added to 2.2 kg wet sediment, without evaporation of the solvent, resulting in 305 mg DEHP/kg (dwt). No effect was observed on the tadpoles in this 'spiking control'. Neither, did the DEHP concentration in lipids extracted from whole animals differ significantly between tadpoles exposed to 300 mg/kg (dwt) in the 'spiking control' compared to tadpoles exposed to the same amount of DEHP with the acetone method. This indicates that the different spiking methods had similar effects on, alternatively did not modify, the bioavailability and the toxicity of the DEHP in Study I. Although, the amount of added ethanol most likely differed between Study I and the 'spiking control' in Study II, these results suggest that the spiking method is not the main reason for the difference in observed effects between the two studies.

b) Sediment character

In Study I, coarse sediment (fragments of leaves and bark) from a small pond was used (P. Larsson, pers. com.), while Study II used a fine-textured lake sediment. Although the organic content was similar in the two sediments, the adsorption capacity of DEHP was probably less in the coarse sediment. This is supported by the higher concentrations of DEHP measured in tadpoles in Study I (ranging between 0.28 to about 25 µg/g (WWT) in sediment exposure concentration up to 500 mg/kg (dwt)) compared to the tadpole concentrations, 0-1.55 µg/g (WWT), measured in Study II. Since moor frogs lay their eggs in shallow ponds or in the littoral of lakes, a coarse sediment like the one in Study I, is more relevant for a natural frog egg/tadpole environment, compared to the more degraded sediments used in Study II.

c) Temperature

The experiments were performed at 5°C in study I and at 10°C in Study II. *R. arvalis* usually lay their eggs when water temperature has reached 10°C, but the temperature in the egg clumps most certainly temporarily dip to < 5°C, e.g. during the nights. During the days, on the other hand, the temperature in the egg clumps increases markedly due to the "greenhouse effect" within the clumps. Eggs of *R. arvalis* are therefore naturally exposed to great variations in temperature.

The most important effect caused by the difference in temperature was probably the time between newly spawned eggs and the hatching of tadpoles (exposure duration), and hence the exposure time when the eggs were in direct contact with the contaminated sediment. In Study I the tadpoles started to hatch after 3 weeks whereas in Study II the corresponding time was 1.5 weeks. Since the duration of embryo contact with the sediment has been identified as an important factor in studies with amphibians (see above, Burton 1992) the difference in duration to hatch between Study I and II might have been the reason for the observed difference in toxic response. In Sweden, the tadpoles of *R. arvalis* normally hatch after 1-1.5 weeks in their natural environments (Berglind pers. com., Andrén. and Nilsson pers. com.), but three weeks in the embryonic stage also occurs, especially for eggs that is laid in the beginning of the egg-laying period (Loman, pers. com.).

Another argument that has been used against the validity of Study I, is the "plateau behaviour" of the dose-response curve. The author (Larsson, pers. com.) gives the following explanation to this: The eggs were aggregated in a clump during the exposure. Therefore, the eggs in the centre were never in direct contact with the contaminated sediment. About the same number of eggs (25-30%) in all treatments, might therefore have been exposed to a much lower degree and hence account for the observed "plateau behaviour".

The facts listed above tell against a rejection of any of the studies in favour of the other.

The third study (Solyom et al., 2001) was performed to explore some of the methodological differences between Studies II and I. In this third study moor frog eggs were exposed to DEHP at two temperatures 5 and 10°C using two different types of sediment, a fine sediment with mostly degraded material (particles > 2 mm 0.1% dwt and TOC 17% dwt) and a coarse sediment containing undegraded material (particles > 2mm 4.7% dwt and TOC 16% dwt). The sediments were spiked with DEHP using essentially the same method as in Study II with acetone as a solvent. The nominal exposure concentrations were 100, 300 and 1,000 mg/ kg dwt. Five beakers with 50 eggs/beaker were used for each test concentration and temperature/sediment combination. Controls with and without acetone were run in parallel. The photoperiod was 12 hours light/12 hours dark. The beakers were gently aerated throughout the study. Temperature O_2 and pH were measured daily and ammonia and nitrite at least once a week in 15 beakers representing all the treatments. The total exposure time was 35 days at 5°C and 26 days at 10°C.

The average measured DEHP concentrations in the sediments were quite variable. Based on measurements at the start and at the end of the study they ranged between 73 and 118% of the nominal concentrations. The exception was the lowest concentration (100 mg/kg nominal) in the test series with fine sediment at 5°C in which the DEHP concentration was 242 mg/kg (242% of nominal).

The median hatching time at 10°C was half of that at 5°C, approximately 12 days compared to approximately 24 days. The hatching success was higher 92-95% at 10°C compared to 73–86% at 5°C. The mortality of the tadpoles ranged from 0 to 3% and the deformation of tadpoles (retarded tail) ranged from 0 to 2.3% at both temperatures. No statistically significant differences between controls and DEHP exposed groups were seen in any of the 4 test series.

Although using the same temperature (5°C) as in study I no effects on egg hatching or tadpole survival were seen in the third study. The reasons for this difference are not clear. The oxygen depletion caused by simultaneous degradation of ethanol and DEHP may be the cause of the dose dependent effects seen in Study I even though this is not supported by the results from the "ethanol" control used by Wennberg et al.. Alternatively the extremely high DEHP concentrations in the water in study I may have caused the effects toxicologically or physically. Despite the fact that the findings in Study I cannot be adequately explained the conclusion drawn based on a weight of evidence is that the hatchability of eggs and survival of tadpoles does not seem to be affected when exposed to DEHP spiked sediments up to 1,000 mg/kg dwt.

3.2.1.3 Toxicity to aquatic invertebrates

The problems with aquatic toxicity testing, caused by the low water solubility of DEHP, is reflected by the inconsistency of the results from the testing on invertebrates and the difficulties in interpreting them.

Formation of microdroplets or surface films at higher test concentrations than the solubility in the actual test media may contribute to effects by direct physical interference. This could lead to an overestimation of the toxicity. One example that has attracted special attention in this context is entrapment of daphnids at the surface, so-called floaters. There is also a risk that the toxicity will be underestimated due to unstable test concentrations where the actual exposure of the organism cannot be determined. This concerns especially static test systems and studies with only nominal test concentrations reported.

Organic material adsorb lipophilic substances and thus increase their apparent water solubility but may also either decrease or increase the bioavailability and thus the toxicity of the substance (Lundberg, 1994). The actual form of DEHP in "solutions" (forming of micelles, microdroplets, surface film, interaction with carrier solvents, adsorption etc.) in various tests using different test concentrations and solvents may thus influence the exposure and the toxicity. However, whether the conditions in a particular test lead to an underestimation or an overestimation of the toxicity does not seem possible to determine today. Judging from those short term and long-term toxicity tests with Daphnids where problems with solubility have been reported, the "apparent water solubility" in the tests seems to be roughly in the order of 0.1mg/l. Above this approximate level test solutions seem not to be stable and solubility-related problems start to arise, e.g. floaters.

The short-term toxicity data of DEHP to aquatic invertebrates are presented in **Table 3.76**. The long-term toxicity data of DEHP to aquatic invertebrates are summarised in **Table 3.77**. Numbers in brackets, [x], used in the text below refer to the numbers the studies have been given in the tables.

Exposure via water

Short-term toxicity tests have been carried out on a variety of invertebrate species from different taxonomic groups. Crustaceans and insects are well represented with several species tested in freshwater, one species in marine water and one in brackish water.

Overall the reported EC_{50}-values range from > 0.046 to > 300 mg/l. Most of the reported EC_{50}-values are "greater than", indicating that no adverse effects were observed at the highest tested concentration. In several of the tests, concentrations showing no effects are much higher than the "apparent water solubility". Most tests are performed in static systems and in many of the studies only nominal test concentrations have been reported i.e. the actual concentration exposing the organisms has not been determined.

There are 5 tests with specific EC_{50}-values reported: 0.133 mg/l [2], 0.33 mg/l [5], 2.0 mg/l [7], 11 mg/l [8], and 16.3 mg/l [18]. No solubility problems (e.g. floaters) are reported from these studies. The lowest value, 0.133 mg/l, is presented in a report that comprises acute toxicity tests with *Daphnia pulex* on 30 compounds (Passino and Smith, 1987). The authors reported that, for some substances, the nominal concentration required to achieve 50% or greater immobilisation of organisms exceeded the solubility, as evidenced by cloudiness, precipitation or a surface film. Substances with solubility problems are noted in a table. DEHP has no such note. The next lowest EC_{50} value, 0.33 mg/l for *D. carinata*, is reported from a study with the aim to compare different toxicity test and organisms regarding their sensitivity, testing a great number of substances (Yoshioka et al., 1987). No problems with solubility of the test substance are mentioned. However, test performance and results are very briefly described in the report.

Long-term toxicity tests have been carried out on several invertebrate species, but studies with *Daphnia magna* as test organism predominate.

1) Sanders et al. (1973) and Mayer and Sanders (1973) reported a 21 days *D. magna* reproduction test in an intermittent-flow system. Reproduction was significantly inhibited at all tested concentrations: 60% at 0.003 mg/l, 70% at 0.010 mg/l and 83% at 0.030 mg/l. However, the reproduction rate was low in this study, 11 offspring per adult. This is lower than the condition for validity in the OECD guideline 202 and could be compared to for instance 170 per adult (Brown and Thompson 1982a) and 200 per adult (Knowles et al. 1987) reported in later studies. The very low effect concentration in this study is also contradicted by the results from all later studies and the result has been determined

as unacceptable by the USEPA after personal communication with the authors of the study. The effect concentration value 0.003 mg/l is considered to be unreliable and for the purpose of this risk assessment it will not be used. Accordingly, later on in this report when referring to number of studies performed and the like, this study will not be included.

2) Knowles et al. (1987) reported a 21-day *D. magna* reproduction test in a flow through system conducted at the same facility as the study by Mayer and Sanders (1973). The reproduction rate in this study was 200 young per adult. Survival and reproduction was not affected in this test at measured concentrations up to 0.158 mg/l (NOEC). At 0.811 mg/l, however, survival was significantly reduced after both 7 and 21 days. The mean number of young per surviving adult was also reduced at this dose level (LOEC). A NOEC of 0.072 mg/l was identified regarding both DNA content and RNA/DNA ratio at day 7 (LOEC 0.158 mg/l). Static biochemical indicators, e.g. DNA, have been shown to be closely associated with total growth. Dynamic biochemical indicators as RNA/DNA ratio has been shown to reflect growth rate. Daphnids were trapped at the surface during the test, but at close examination they appeared to be feeding and healthy, the authors noted. The number of floaters was dependent on both dose and time. The lowest concentration where the number of floaters significantly differed from the control was 0.158 mg/l at day 0. By day 21 floaters were observed only at 0.811 mg/l.

3) Chronic toxicity of DEHP to *D. magna* was estimated in a test program comprising 14 phthalate esters (Springborn Laboratories (1984c), Cox and Moran (1984), Rhodes et al. (1995)). The 21-day reproduction test was conducted in a flow through system. Survival was the most sensitive end-point studied, with a NOEC-value of 0.077 mg/l and a LOEC-value of 0.16mg/l, measured concentrations. Reproduction was a less sensitive end-point showing no significant reduction at the highest tested concentration, 0.29 mg/l. The reproduction rate ranged from 56 to 116 mean offspring per adult in the tests of the 14 phthalate esters. Mean offspring per adult was 56 in the DEHP test. At 0.29 mg/l daphnids were observed entrapped on the surface throughout the study. At 0.16 mg/l daphnids were observed entrapped on the surface day 7 and 14, but not day 21. At 0.077 mg/l daphnids were observed entrapped on the surface day 7, but not day 14 and day 21. In the control daphnids were observed entrapped on the surface throughout the study. According to the authors the cause of entrapment in the control solutions is unknown; however, this entrapment did not affect the daphnids survival or reproduction (Springborn Laboratories, 1984c).

4) In a 21-day *D. magna* reproduction test under semistatic conditions Brown and Thompson (1982a) found no effect on survival or reproduction up to the highest tested nominal concentration of 0.1 mg/l. The reproduction rate in this study was approximately 170 young per adult. In the reproduction test (≤ 0.1 mg/l) no floating Daphnia was reported. In an acute toxicity test presented in the same paper Daphnia floated on the surface after 48 hours, 25% at 0.169 mg/l and 100% at 0.304 mg/l; mean measured concentrations. The authors also found from a solubility/stability-test that DEHP at levels below 0.18 mg/l result in stable solutions, whereas above this level loss of phthalate from the bulk solution occurs.

5) Adams and Heidolph (1985) reported significant effects on both survival and reproduction at 1.3 mg/l, in a 21-day *D. magna* reproduction test under semistatic conditions. NOEC was determined to be 0.64 mg/l. The reproduction rate in this study is unknown. No floaters are mentioned in the report.

6,7) Brown et al. (1998) presented a report including four chronic toxicity studies with *D. magna* on 13 phthalate esters using dispersants. DEHP was tested in two of the studies in 21-day *D. magna* reproduction tests under semistatic conditions.

In one of the studies, using "Tween 20" as dispersant, two DEHP concentrations were tested; 0.25 and 1.0 mg/l. End-points were reproduction, survival and length of surviving adults. At both concentrations there was a reduction in numbers of offspring produced relative to the dispersant control. The reduction was significant at 0.25 mg/l but not at 1 mg/l. Relative to the control without dispersant the reduction in numbers of offspring was not significant in any of the concentrations. There was no significant mortality in the test. At both concentrations there was a reduction in length relative to the control without dispersant. The reduction was significant at 0.25 mg/l but not at 1 mg/l. Relative to the dispersant control the reduction in length was not significant in any of the concentrations.

In the other study, using "Marlowet R40" as dispersant, only one single DEHP concentration was tested, 1.0 mg/l. End-points were reproduction, survival and length of surviving adults. In this study no significant adverse effects were observed relative to dispersant control or the control without dispersant.

The reproduction rate in the controls in the phthalate studies ranged from 93 to 167 mean offspring per adult. No floaters were observed in the studies.

In all four studies presented in the report there appeared to be a promotion in the numbers of offspring in the dispersant control relative to the control without dispersant. This promotion was only significant in a study using "Marlowet R40" as dispersant where DEHP was not tested. Separate studies with the dispersants confirm the observation that "Marlowet R40" promotes the number of young produced at a concentration of 10 mg/l, the same dispersant concentration as used in the DEHP tests along with the DEHP concentrations of 1 mg/l. For "Tween 20" the results do not confirm that this dispersant also promotes the numbers of young at 10 mg/l. "Tween 20" was toxic to *D. magna* reproduction at 32 mg/l.

8) In a 21-day *D. magna* semistatic reproduction test using the dispersant Marlowet R 40 as solubiliser Scholz (1995b) found no adverse effect on survival or reproduction up to the highest tested concentration of 14 mg DEHP/l. The reproduction rate was approximately 70 young per adult in the control and 100 in the test concentrations. The dispersant, Marlowet R40, obviously increased the number of offspring. The reproduction rate was significantly higher in all test concentrations and in the solubilizer control, compared to the control without solubiliser. No floaters are mentioned in the report.

9) The effects of DEHP on overall locomotor activity of *Gammarus pulex* was studied by Thurén and Woin (1991). The amphipods were exposed to DEHP at concentrations of 0.1 and 0.5 mg/l for 10 days under flow through conditions. There was a 5-day pre-exposure and a 10-day post-exposure period. The overall locomotor activity was significantly decreased at the higher exposure level, and the effect persisted throughout the post-exposure period. No significant effects were observed at the lower dose level. The authors commented that the DEHP adsorbed to and accumulated by the organisms could have caused organs sensitive to water velocity, and "olfactory" organs to malfunction, thereby reducing the mobility and affecting upstream movement. The decreasing activity following DEHP exposure could therefore have been the result of mechanical and/or physiological effects.

10) The sediment-dweller *Chironomus plumosus* was exposed to DEHP added to water, but with sand or hydrosoil present in the test system (Streufert, 1977; Streufert et al., 1980). No effects were observed at the highest concentrations, 0.36 and 0.24 mg/l respectively, measured after 35-40 days of exposure.

11) Laughlin et al. (1978) reported a 22 days semistatic toxicity test with larvae of grass shrimps, *Palamonetes pugio,* as test organisms. They found no adverse effect on survival or development rate up to the highest tested nominal concentration of 1 mg/l. At the highest concentration, small droplets of DEHP were sometimes observed and a considerable loss of substance could be shown within 24 hours, for all three esters tested.

12) Brown and Thompson (1982b) reported a bioconcentration study with blue mussels, *Mytilus edulis,* as test organisms, that also have been referred to as a toxicity test. The exposure period was 28 days followed by a depuration period of 14 days. No adverse effects were observed at the highest nominal test concentration of 0.05 mg/l (mean measured concentration 0.0421 mg/l).

As regards long term toxicity to invertebrates exposed via water the reported NOEC-values thus range from 0.072 to 14 mg/l. Several of the reported LOEC-values are "greater than", meaning that no adverse effects were observed at the highest tested concentration. In some of the tests, using dispersants, concentrations that show no effects are much higher than the "apparent water solubility". However, there are three Daphnia reproduction tests showing effects at or below the highest tested concentration with specific NOEC (and LOEC) values reported; 0.072 (0.158) mg/l [2], 0.077 (0.16) mg/l [3], and 0.640 (1.30) mg/l [5].

An overall conclusion regarding the toxicity to aquatic invertebrates exposed via water in terms of specifying a NOEC-value for use in the risk assessment is bound up with problems. There are several indications that the effects observed in the toxicity tests with Daphnia could be caused by physical effects, which probably have no relevance in the environment. There are also indications that DEHP has no shown genuine toxic effect in concentrations up to and markedly exceeding the water solubility (neither the "true" solubility predicted from the physico-chemical properties nor the "apparent" solubility found in some toxicity tests):

- In three Daphnia acute toxicity tests using dispersants (Tween 20, Marlowet R40) no significant adverse effects were observed at the highest tested concentration (1, 1 and 100 mg/l), well above the water solubility.
- In three Daphnia reproduction tests using dispersants (Tween 20, Marlowet R40) no significant adverse effects were observed at the highest tested concentration (1, 1 and 14 mg/l), well above the water solubility.
- In one (two test protocols) of the five Daphnia acute toxicity studies showing effects at or below the highest tested concentration and not using dispersants, floaters have been reported, indicating that the effects may be physical.
- In two of the three Daphnia reproduction tests showing effects at or below the highest tested concentration and not using dispersants, floaters have been reported, indicating that the effects may be physical.
- Floaters may have been present, although not reported, in other daphnia studies possibly causing adverse effects.

  However, there are also several arguments against taking the view that all effects observed in Daphnia toxicity studies should be of physical nature.

- There are only two Daphnia acute toxicity studies and two Daphnia reproduction tests with floaters reported. This is a weak basis for assuming that floaters were present but not reported, also in the other studies.
- There are studies with adverse effects observed but no floaters reported at approximately the same levels of concentration as the ”practical” water solubility found in some toxicity tests (roughly about 0.1 mg/l) and also at concentrations clearly above this level:
  - Four Daphnia acute toxicity tests, EC_{50} 0.133, 0.33, 2.0 and 11 mg/l.
  - One Daphnia reproduction test, NOEC 0.64 mg/l (LOEC 1.3 mg/l)
- It is not obvious that there is a causal connection between floaters and the observed adverse effects in the studies reported:
  - There are only two Daphnia reproduction tests with floaters reported. In both tests floaters have been observed at the concentrations where the adverse effects were observed. However, in one of the tests floaters are also reported in the control during the whole test without any adverse effects observed. In the other study the authors noted that the daphnids that are trapped at the surface appeared to be feeding and healthy.
  - There are only two Daphnia acute toxicity studies with floaters reported. In one of them, there are two tests reported where all daphnids died, at exposure concentrations exceeding 1 mg/l. In the other study no adverse effects where observed, despite that 25% of the daphnids where observed to be floaters at 0.169 mg/l and 100% at 0.304 mg/l.
- The tests using dispersants do not provide conclusive evidence that DEHP has no genuine toxic effect in concentrations up to and markedly exceeding the water solubility.
  - The absence of adverse effects at a certain concentration in one test does not in itself show that effects reported in another test at the same or a lower concentration level should be erroneous. To reject an observed effect requires a high level of proof. For example, if the result is an outlier and a substantial number of test-results from the same test organism, under similar conditions, points to another level of toxicity. Alternatively, that a convincing causal explanation of the differences between studies shows the one representing pronounced higher toxicity to be erroneous and the others to be representative.
  - The interaction between DEHP and the dispersants in the tests and how the dispersants possibly influence the uptake of DEHP does not seem clear.
  - The dispersants themselves seem to influence Daphnia reproduction positively.
  - There are significant adverse effects shown at 0.25 mg DEHP/l in one of the chronic toxicity tests using dispersants.

All the uncertainties, the great variation in the results and the difficulties to interpret the results regarding the DEHP toxicity to aquatic invertebrates makes it difficult to choose a precise NOEC-value from a specific test to use as part of the basis for derivation of a PNEC for water in the risk assessment. Depending on the approach chosen there are several options how to handle these uncertainties/ difficulties:

- By way of precaution it might be appropriate to choose the lowest NOEC-values found in long term toxicity tests, despite that floaters have been noted in the two Daphnia magna

reproduction tests showing the lowest NOEC-values; 0.072 mg/l and 0.077 mg/l (Study 2 and 3 in **Table 3.77**). These NOEC-values may also be supported by the EC_{50}-values of 0.133 mg/l, 0.33 mg/l and 2.0 mg/l in acute toxicity studies with *Daphnia pulex*, *D. carinata* and *D. magna* respectively (study 2, 5 and 7 in **Table 3.76**).

- Another precautionary approach might be a reasoning based on the fact that several tests show effect or no effect level roughly about 0.1 mg/l (Study 2 and 5 in **Table 3.76** and study 2, 3, 5, 7 and 9 in **Table 3.77**). This also seems to be the approximate level for "apparent water solubility" of DEHP in several toxicity tests. Until more elucidative tests handling the solubility problems with DEHP prove otherwise the value 0.1mg/l could be legitimate to use as NOEC for aquatic invertebrates.
- An approach of less precaution could be to consider all the effects reported physically caused and not relevant, with the conclusion that DEHP has no (shown) toxicity up to and markedly exceeding concentrations possible to achieve in natural waters.
- Finally, one might conclude that it is impossible from the current data to determine whether any effects observed in the toxicity tests may be relevant to use for derivation of a PNEC for water. Until more elucidative tests handling the solubility problems with DEHP are performed no value could be set to use as NOEC for aquatic invertebrates.

It appears highly uncertain whether floaters actually were present in all tests at the test concentrations showing effects. If present, it is also unclear if the floating (or other physical interference) is the actual cause of the effects reported. Based on the present data it is considered not feasible to determine a level of toxicity for DEHP to aquatic invertebrates exposed via water. Accordingly, for the purpose of this risk assessment the last option is considered legitimate to use. Hence, it is not possible, for the time being, to state a $NOEC_{water}$ for aquatic invertebrates.

Exposure via sediment

Guidelines exist for sediment-tests with the amphipod *Hyalella azteca* and for midge larvae, *Chironomus* spp. (e.g. ASTM, 1993). A test duration ≤ 10 days, for these test-organisms, is considered as a short-term test (*ibid*). According to Hill et al. (1994) tests with chironomids should be started using 1st or 2nd instar larvae. Midge exposures starting with older larvae may underestimate midge sensitivity to toxicants (Burton, 1992). *Chironomus tentans* and *Hyalella azteca* were exposed for 10 days (short-term) to DEHP-contaminated sediment. No effect was observed at the highest concentration used approximately 3,000 mg/kg (dwt) (Call, 1997).

There are two long-term studies with aquatic invertebrates exposed to DEHP via contaminated sediment. One study (Thompson et al., 1995, Brown et al., 1996) was performed with the midge *Chironomus riparius* in good accordance with available guidelines (ASTM, 1993). DEHP in acetone (4 ml) was added to a dried sediment portion and the solvent was evaporated before the spiked portion was blended into wet sediment (resulting in a sediment portion with a total dry weight of 170 g). No effect was observed on hatching and survival of the midges at the highest tested concentration, 11,000 mg/kg (dwt).

In another study (Woin and Larsson, 1987), predatory dragonfly larvae were kept in aquaria for 60 days, exposed to DEHP contaminated sediment (DEHP added with 100 ml ethanol directly into 10 litres of wet sediment). The sediment was left for 3 days to equilibrate with the phthalate before water and larvae were added. The predation efficiency was 'significantly affected' by 15-25% compared to solvent control (containing 1 mg DEHP/kg (WWT)) at about 600 mg/kg (WWT) (LOEC), the only concentration tested. According to TGD a NOEC can be calculated as LOEC/2 when the effect percentage is 10-20%, resulting in a NOEC of about 300 mg/kg

(WWT). Applying the factor of 2.6 set by TGD for converting sediment concentration from wet weight to dry weight results in a NOEC of 780 mg/kg (dwt). However, the results from this study are considered less reliable due to only one test concentration and the high amounts of ethanol used when spiking the sediment.

The $NOEC_{sediment}$ values for detritivorous and predatory invertebrates are > 11,000 and 780 mg/kg (dwt) respectively.

3.2.1.4 Toxicity to algae and higher plants

The toxicity data of DEHP to algae and higher plants are summarised in **Table 3.78**.

No measured LOEC values are available for algae or higher plants, only nominal effect concentrations and "greater than" values. The long-term (7d) nominal EC_{50}-values between 397 and 7,582 mg/l, reported for the macrophyte *Lemna gibba* (Davis, 1981) are several orders of magnitude above the apparent solubility of DEHP. The actual effect concentrations in these tests were therefore most likely lower than the reported. In addition, the comment that the toxicant formed oil droplets etc. further supports that the actual concentration of dissolved DEHP in this study, was considerably lower than the nominal values. This might also indicate that the observed effects were due to physical effects, not relevant for environmental exposure conditions.

The LOEC, 10 mg/l, reported for *Scenedesmus quadricauda* (Bringham and Kühn, 1980) and the EC_{50} value, 30g/L, reported for the marine algae, *Gymnodinium breve* (Wilson et al., 1978) are also far above reported solubility for DEHP. The actual effect concentrations are therefore, also in these studies, probably lower than the reported nominal values and/ or the observed effects might be due to physical interference.

Two studies exist with measured concentrations. In both these studies only one concentration of DEHP was tested, and no effects were observed at these test concentrations, compared to the controls:

a) In one study *Scenedesmus subspicatus* was exposed to 130 mg/l in the presence of the solubilizer MARLOWET R 40 (Hüls AG, 1995). The solubiliser might have affected the availability of DEHP. This is not discussed in the report and hence the NOEC cannot be used as a basis for PNEC.

b) *Selenastrum capricornutum* was exposed to 0.1 mg/l (NOEC) without the use of a vehicle (Adams et al., 1995). No LOEC was obtained.

From studies of toxicity on algae and higher plants it can only be concluded that it is impossible from the current data to determine whether any effects observed in the toxicity tests may be relevant to use for derivation of a PNEC for water.

Hence, from the available data, it is not possible to state a $NOEC_{water}$ for algae and higher plants.

3.2.1.5 Toxicity to micro-organisms

The toxicity data of DEHP to micro-organisms are summarised in **Table 3.79**.

Several different types of toxicity studies involving micro-organisms have been carried out with DEHP. These include single species tests, tests on sediments as well as on sludge from sewage

treatment plants (STP), and mesocosm studies. According to TGD results from model ecosystems can be used in the risk assessment and should be reviewed on a case by case basis. Three groups of studies have been identified. Each group are summarised separately in the survey below: i) those in which natural communities from water compartments or single test-species are exposed via water ii) those in which natural benthic communities are exposed via contaminated sediment and iii) studies with sludge from STPs.

Exposure via water

There is only one study with micro-organisms in water, where the test concentrations were measured. In a marine model ecosystem, no effects were observed at the highest DEHP concentrations tested, 0.059 mg/l during winter and 0.016 mg/l during summer (Perez et al. 1983) (It should be noted that the marine conditions might have affected the bioavailability of the substance).

Threshold concentrations (when effects start to occur, 3-5% growth inhibition) ranging between 19 and 53 mg/l are reported for three protozoa species (Bringmann and Kühn 1980, 1981). The threshold concentrations are extrapolated from regressions between the (probably nominal) NOEC and LOEC concentrations. However, the NOECs and LOECs are not reported. Neither are the effect levels at higher concentrations. The extrapolated threshold concentrations are all far above the apparent solubility level for DEHP, and the observed effects might be due to exposure situations (e.g. aggregates of DEHP) which have no relevance for the environment. Hence, these data cannot be used in order to derive a NOEC for protozoa.

Pseudomonas putida was exposed to DEHP in three different tests. In one of the studies 20% inhibition of respiration was observed at concentrations between 19 and 2500 mg/l, but not at higher concentrations (BASF AG, 1991). This study is therefore considered invalid. In the other two studies an EC_{10} at the nominal concentration of 1,671 mg/l (in the presence of solubiliser) (Hüls AG 1996) and a nominal NOEC at the highest tested concentration of 400 mg/l (Bringmann and Kühn 1980) are reported.

Since only nominal concentrations were reported in the studies where effects were observed, a NOEC- level for micro-organisms in water cannot be determined.

Exposure via sediment

Three unconventional studies deal with the effects of DEHP on natural microbial flora in sediment.

Mesocosm studies are more likely to reflect reality, than are shorter laboratory studies. In a marine mesocosm study 34% reduced ammonia flux from the benthic compartment was observed at a sediment concentration of about 6.2 mg DEHP /kg (dwt) in the uppermost 7 cm of the sediment (Perez et al. 1983). The effect was observed after 30 days of exposure, during summer conditions. The next lower concentration was 0.2 mg/kg (dwt). In their discussion, Perez et al. remark that a more realistic effect concentration, causing the reduced ammonia flux, would be that of the uppermost 3 cm in the sediment where the concentrations were approximately three times higher than the average concentration in the 0-7 cm layer. Therefore, for the purpose of this risk assessment, a LOEC of 18.6 mg/kg (dwt) and a NOEC of 0.6 mg/kg (dwt) was derived by multiplying the reported LOEC/NOEC values for the 0-7 cm layer by a factor of about three. Weaknesses with this study are that there is no precise description of the effect parameter used, and that the interval between the measured sediment concentrations is larger than recommended (approximately 30 times). Therefore, the results from this study are considered less reliable.

Larsson et al. (1986) observed reduced respiration rates in sediment cores spiked with DEHP. Twenty hours of exposure resulted in a LOEC (17% inhibition of oxygen consumption) at 33.4 mg/kg (WWT). According to TGD a NOEC can be derived from LOEC/2 if the observed effect level is between 10 and 20%. When applying this on the LOEC above a NOEC of 16.7 mg/kg (WWT) is obtained. Application of a wet weight to dry weight conversion factor of 2.6 (TGD) on these values results in a NOEC of 43 mg/kg (dwt) and a LOEC of 87 mg/kg (dwt). These values were based on measured exposure figures that were calculated with the assumption that all the added DEHP was evenly distributed in the uppermost five centimetres of the sediment core. However, since the DEHP was injected 0.5 cm below the sediment water interface, a concentration gradient with highest concentrations near the sediment surface was probably the actual case in the analysed 5 cm layer. This may lead to an underestimation of the actual exposure concentrations of approximately one order of magnitude. Therefore, the estimated NOEC above cannot be used in the risk assessment. However, the results can be used as a support to the study above, in the sense that DEHP may affect microbial processes in sediment.

A third briefly presented study showed no effect on freshwater sediment flora at a DEHP concentration of 100 mg/l fresh sediment (Mutz and Jones, 1977).

To summarise these three sediment studies, the lowest $NOEC_{sediment}$ was about 0.6 mg/kg (dwt) and the corresponding $LOEC_{sediment}$ was 18.6 mg/kg (dwt). This interval, between the NOEC and LOEC values, was larger than recommended. In addition the actual exposure concentrations were calculated from imprecise assumptions. Therefore, no firm conclusions can be drawn from these studies regarding effects on microbially mediated processes in the sediment compartment.

Exposure via sludge

All results available for effects on microorganisms in STP sludge are based on nominal concentrations, above the solubility level for DEHP in distilled water. However, the solubility of DEHP in sludge mixtures is most likely higher than in distilled water, due to the association of the substance to dissolved (organic) compounds. Since the bacteria are supposed to grow on the organic material in the sludge-sewage mixture, they are also exposed to chemicals associated with this material. In addition, Gibbons and Alexander (1989) showed that bacteria that are able to grow on DEHP excrete products that increase the solubility of DEHP. Hence, in toxicity tests with STP sludge inoculum, it is not irrelevant to use exposure concentrations exceeding the molecular as well as the "apparent" solubility for DEHP.

Two studies indicate that DEHP might affect anaerobic processes in STPs, one study showing suppression of the methanogenesis after 26-day exposure to DEHP (O'Connor et al., 1989), and one "fermentation tube test" only accounted for in the IUCLID database. The reported NOECs are 100 and < 10-20 mg/l respectively. The NOEC from the latter study cannot be used since no further information on the study has been made available to us (the Industry claims that the method used in the study "was not a satisfactory procedure" and "should not be considered valid for the purpose of the risk assessment").

The effect of DEHP on respiration in activated sludge was tested in three studies. In one case the respiration was reduced by 14% at 0.4 mg/l (LOEC), compared to control (Volskay and Leslie Grady, 1988). The origin of the activated sludge was not reported. Since a test is considered valid if the respiration rates of the controls are within 15% of each other (according to the OECD guideline 209) an effect level of 14%, with only one concentration tested, cannot be considered a significant effect. Therefore this study cannot be used in order to establish a NOEC for the risk assessment. In another study, which was performed with activated sludge from an industrial (BASF) treatment plant, no effect was observed at the highest concentration tested, 1,960 mg/l

(BASF AG 1983). This sludge was probably preadapted to DEHP, and the study is therefore considered invalid in order to establish a NOEC for this RAR. In a recent study (Hüls Infracor, 1999), DEHP was added both directly and with emulsifier (TWEEN 80) to activated sludge from the municipal treatment plant Marl-West, treating predominantly domestic sewage. No inhibition of activated sludge respiration was observed at the highest tested DEHP concentrations, 2,007 mg/l (without emulsifier) and 1,000 mg/l (with TWEEN 80) respectively (no explanation is given why different max. conc. were used). We consider the test without emulsifier as most relevant. Hence, the lowest available NOEC for respiration is (>) 2,007 mg/l.

An overall conclusion: Most of the reviewed studies above, on effects of DEHP on microorganisms in different media indicate that DEHP is not harmful to microorganisms. However, a couple of the studies indicate that DEHP might affect especially anaerobic bacteria/ processes in sediment and also in sludge at long-term exposure (e.g. Perez et al., 1983; O'Connor et al., 1989; and the unpublished "fermentation tube test" from IUCLID). Effects (non-significant) on *Pseudomonas* were also indicated in soil tests (see Section 3.2.3.3).

3.2.1.6 PNEC Water

Short term and/or long-term effect studies, where the test organisms are exposed to DEHP via water, are available for fish, amphibians, aquatic invertebrates, algae, higher plants, and micro-organisms. However, there are no reliable long-term studies below the apparent water solubility of DEHP indicating effects on organisms exposed to DEHP in water. Therefore, it is not considered suitable to specify a chronic NOEC for organisms exposed via water.

Hence a $PNEC_{water}$ cannot be specified.

However, there are studies showing effects of DEHP when fish are exposed via the food. A NOEC of 160 mg/kg food (ww) can be derived from two studies where effects of DEHP (administered via food) on gonadal development of Atlantic salmon were found (Norrgren et al., 1999, Norman et al.). TGD does not give any guidance on how to use results from studies were the test organisms have been exposed via the food only. However, as food probably is the most relevant exposure route for fish this NOEC will be used in the risk assessment. Applying an assessment factor of 10 leads to a $PNEC_{food}$ of 16 mg/kg (fresh food).

3.2.1.7 PNEC Sediment

Standardised sediment tests are under development and therefore no specific method has so far been specially recommended by TGD for use in risk characterisation, but greater weight should normally be attached to studies carried out according to current methods (e.g. EC, OECD, or EPA) (TGD). However, based on an ad-hoc sub-group meeting on sediment toxicity testing at TM IV (Nov. 1998), an ongoing revision work of TGD was initiated. In this work long-term tests with *Chironomus riparius* / *C. tentans*, *Lumbriculus variegatus* / *Tubifex tubifex* and *Hyalella* / *Gammarus* are recommended as test organisms, representing different habitats and feeding strategies (Minutes: TM IV, '98, Annex 8).

Several studies, mostly applying unconventional test methods, exist in which organisms are exposed to DEHP in sediment. In these studies, which involve organisms from several trophic levels (micro-organisms, detritivorous and predatory insect larvae, and amphibians), different methods have been used in order to distribute the DEHP homogeneously into the sediments.

Since no unanimous guidance exists on how to spike poorly soluble substances into sediments, none of the studies have been invalidated due to the spiking method.

In TGD it is presumed that sediment toxicity data does not exist, hence, no guidance is given how to use assessment factors on such data. In the ongoing revision work of TGD it has been proposed that an AF of 10 can be used when three long-term tests with species representing different living and feeding conditions are available (draft Technical Recommendation, TGD, Chapter 3, Section 5.2). For the water compartment "micro-organisms representing a further trophic level may only be used if non-adapted pure cultures was tested" according to TGD. We consider that the sediment compartment is an especially important habitat for micro-organisms, and think that tests with a natural micro-organism population could be regarded as representative for a further trophic level.

The available studies with sediment-dwelling organisms exposed to DEHP show largely varying results. Short-term and long-term tests with *Chironomus spp.* larvae did not result in any effect at the highest concentrations tested, 3,000 (the only concentration tested) and 11,000 mg/kg (dwt) respectively. The latter value represents the $NOEC_{sediment}$ for midges (detritivorous invertebrates). The more unconventional studies indicated a $NOEC_{sediment}$ for predatory invertebrates of 780 mg/kg (dwt), derived from the study with dragonfly larvae. However this study is considered less reliable and is only used as an indication that effects may occur, since only one concentration was tested. For amphibians, a $NOEC_{sediment} > 1{,}000$ mg/kg (dwt) was obtained. Studies with natural benthic micro-organisms flora indicate that effects on microbially mediated processes might occur at sediment concentrations around 1mg/kg (dwt) (see Section 3.2.1.5).

These NOEC values indicate that the midge larvae are less sensitive than other benthic species. This hypothesis is supported by a summary of acute toxicity studies with nine different organisms exposed to 14 different phthalate esters via water. It appeared from this study that the midge *Paratanytarsus parthenogenetica* was less sensitive to phthalate esters than were species from other trophic levels (Adams et al., 1995). Södergren (1982) showed in static model ecosystems, with addition of ^{14}C-labelled DEHP that the bioaccumulation factor for *Chironomus* sp. was low (up to 20 times lower) compared to BAFs for other benthic organisms. Therefore, it seems likely that midge larvae are less sensitive than other species to exposure of sediment associated DEHP.

Although many questions remain regarding the different sediment studies, the results taken together indicates that effects of DEHP in the sediment compartment may arise at concentrations around 1mg/kg (dwt). However, the results from the micro-organism studies are considered less reliable due to methodological uncertainties and will therefore not be used to derive a PNEC sediment. Furthermore, studies on microorganisms in sludge and soil (see Setion 3.2.3.3) do not indicate effects at such low exposure levels. The NOEC of > 1,000 mg/kg derived from the frog studies is chosen for the derivation of a $PNEC_{sediment.}$ Effect studies exist with organisms from three (four?) trophic levels. Therefore an assessment factor of 10 is used, resulting in a PNEC of >100 mg/kg (dwt).

Since no $PNEC_{water}$ could be determined, the equilibrium partitioning method, given in TGD, cannot be used to estimate $PNEC_{sediment}$.

$$PNEC_{sediment} = > 100 \text{ mg/kg (dwt)}$$

3.2.1.8 PNEC for micro-organisms in STP

According to TGD the $PNEC_{micro\text{-}organisms}$ is set equal to a NOEC from a test performed with specific bacterial populations like nitrifying bacteria and *Pseudomonas putida*. (It is however stated in TGD that results from the cell inhibition test with *P. putida* "should be treated with care" when used for affect assessment for STP). When this is applied on the results discussed above for *P. putida* a PNEC > 400 mg/l is obtained.

The Technical Recommendation for TGD (Doc. ECB4/TR1/98, Chapter 3, Section 4) recognises the importance of - especially ciliated - protozoa for the functioning of conventional STPs. "The function of the protozoa in STP is correlated to their growth". It is therefore stated that growth inhibition tests with ciliated protozoa are relevant for the risk assessment for STPs. There are studies available indicating that DEHP causes growth inhibition of protozoa, at concentrations >19 mg/l.

However, both the results for protozoa and for *P. putida* are based on single species tests in water from which only nominal (?) NOECs, far above the apparent solubility level, are reported. Therefore, they cannot be used in order to derive a $PNEC_{STP}$.

Also in studies carried out with activated sludge, only nominal concentrations were reported. However, these studies were considered valid since the solubility of DEHP in such tests is most likely higher than in clean water, due to the association of the substance to dissolved (organic) compounds. The results from such tests indicated effects on the anaerobic processes A NOEC at 100 mg/l was obtained for suppression of methanogenesis. This result was obtained in a 26 days test and it is stated in TGD that "short-term measurements in terms of hours (e.g. 10 hours) are preferred, in accordance with the retention time in a STP". Therefore, this study is not used in order to obtain a $PNEC_{STP}$. However, it indicates that the fermentation step in the treatment of sewage sludge could be affected by high concentrations of DEHP in the sludge.

Only one study on respiration in activated sludge was considered valid for the risk assessment. The NOEC from this study was 2,007 mg/l (highest tested conc.).

To summarise, according to TGD only the NOEC for respiration in activated sludge can be used to obtain a $PNEC_{STP}$ for the purpose of this risk assessment. According to TGD an assessment factor of 10 should be used on NOECs obtained from respiration tests. Hence, the $PNEC_{STP}$ is > 201 mg/l.

Table 3.71 Short term toxicity to fish

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint,	Effect conc. (mg/l)		Comment	Reference
Pimephales promelas	20°C St M		96 hours	Mortality	LC_{50}	> 0.16		Adams et al. (1995)
Cyprinidon variegetus Ma	25°C Fl M		96 hours	Mortality	LC_{50}	> 0.17		Springborn Bionomics (1984a) Adams et al. (1995)
Lepomis macrochirus	St		96 hours	Mortality	LC_{50}	> 0.20		Adams et al. (1995)
Oncorhynchus mykiss	11°C Fl M		96 hours	Mortality	LC_{50}	> 0.32		EG and G Bionomics (1983a) Adams et al. (1995)
Brachydanio rerio	Se		96 hours	Mortality	LC_{50}	> 0.32	in Dutch	Canton et al. (1984)
Jordanella floridae (larvae 1-2 d, juvenile 4-5 w)	23±2°C Se		96 hours	Mortality	LC_{50}	> 0.32	in Dutch	Adema et al. (1981)
Oryzias latipes (larvae 1-2 d, juvenile 4-5 w)	23±2°C Se		96 hours	Mortality	LC_{50}	> 0.32	in Dutch	
Poecilia reticulata	23±2°C Se		96 hours	Mortality	LC_{50}	> 0.32	in Dutch	
Gasterosteus aculeatus	19±1°C Se		96 hours	mortality	LC_{50}	> 0.32		Van den Dikkenberg (1989)
Lepomis macrochirus	22°C St		96 hours	mortality	LC_{50}	> 0.32		EG and G Bionomics (1983b)

Table 3.71 continued overleaf

Table 3.71 continued Short term toxicity to fish

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint,	Effect conc. (mg/l)		Comment	Reference
Pimephales promelas	T ? Fl		96 hours	mortality	LC_{50}	> 0.327		DeFoe et al. (1990)
Oryzias latipes	T? Fl		96 hours	mortality	LC_{50}	> 0.67		
Oncorhynchus mykiss	T? Fl		96 hours	mortality	LC_{50}	> 19.5		
Pimephales promelas	22°C Fl M		96 hours	mortality	LC_{50}	> 0.67		EG and G Bionomics (1983c) Adams et al. (1995)
Oryzias latipes	20±1°C St		48 hours	Mortality	LC_{50}	-	Test concentrations not reported. The study comprised toxicity tests on 22 substances with four organisms for comparison. The LC_{50} is reported to be higher than the highest tested concentration (≥ saturation). Solubility reported to be < 1 mg/l.	Yoshioka et al. (1986)
Pimephales promelas	T? St		96 hours	Mortality	LC_{50}	> 5		Waggy and Payne (1974)
Ictalurus punctatus	T? St		96 hours	Mortality	LC_{50}	> 10		Mayer and Sanders (1973)
Lepomis macrochirus	T? St		96 hours	Mortality	LC_{50}	> 10		
Pimephales promelas	T? St		96 hours	Mortality	LC_{50}	> 10		
Salmo gairdneri	T? St		96 hours	Mortality	LC_{50}	> 10		
Salmo salar			96 hours	Mortality	LC_{50}	> 10	No mortality. No details given on experimental procedure.	Zitko (1972)

Table 3.71 continued overleaf

Table 3.71 continued Short term toxicity to fish

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint,	Effect conc. (mg/l)		Comment	Reference
Brachydanio rerio	20±2°C Se M	Marlowe t R 40 100mg/l	96 hours	Mortality	LC_{50}	> 100	One test concentration . No mortality	Menzel (1995)
Ictalurus punctatus	20°C St N?		96 hours	Mortality	LC_{50}	> 100	No information on test procedures. Only LC50-value given.	Johnson and Finley (1980)
Lepomis macrochirus	17°C St N?		96 hours	Mortality	LC_{50}	> 100	No information on test procedures. Only LC50-value given.	
Oncorhynchus kisutch	16°C St N?		96 hours	Mortality	LC_{50}	> 100	No information on test procedures. Only LC50-value given.	
Salmo gairdneri	12°C St N?		96 hours	Mortality	LC_{50}	> 100	No information on test procedures. Only LC50-value given.	
Lepomis macrochirus	18±0.5°C St N		96 hours	Mortality	LC_{50}	> 250	No mortality at highest tested concentration.	Bionomics INC. (1972)
Oncorhynchus mykiss	15±1°C St N	emulsifie d by mechani cal means	96 hours	Mortality	LC_{50}	540	The authors postulate that the test emulsions may have killed primarily by means of a physical coating action on the fish. Dead fish were coated, possibly by the test compound and fish were observed to stay near the surface and cough throughout the test.	Hrudey et al. (1976)
Cyprinidon variegetus Ma	T? St N		96 hours	mortality	LC_{50}	> 550	No mortality at highest tested concentration.	Heitmuller et al. (1981)
Salmo gairdneri			48 hours	Mortality	LC_{50}	> 1,000	In Finnish. No mortality	Silvo (1974)

Table 3.71 continued overleaf

Table 3.71 continued Short term toxicity to fish

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint,	Effect conc. (mg/l)		Comment	Reference
Oryzias latipes	T? St or Se N		48 hours	Mortality	LC_{50}	> 3,000	No information on mortality.	Ministry of International Trade and Industry (1992)

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations

Table 3.72 Chronic (long term) toxicity to fish exposed via the water

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Pimephales promelas (7.5 months)	25ºC Fl M	Acetone 0,25 ml/l	56 days	Mortality growth	NOEC	> 0.062	test conc. 0, 0.0019, 0.0025, 0.0046, 0.0081, 0.014, 0.030, 0.062 mg/l	Mehrle and Mayer (1976)
Oryzias latipes (larval 1- 3 d)	25 ±1ºC Fl		168 days	Growth survival	NOEC	< 0.554	significant (p= 0,05) weight reduction, 13,4%, when compared to control No effects on survival	DeFoe et al. (1990)
Poecilia reticulata (juvenile 3-4 w)	? ? ?	DMSO 0.1ml/l	35 days	Mortality growth	NOEC	> 0.32		Adema et al. (1981)
Salvelinus fontinalis (adult 1.5 year)	9-15ºC Fl N	Acetone Conc.?	150 days	reduced vertebral collagen levels increased hydroxyproline levels in collagen	LOEC NOEC	0.0037 < 0.0037	No effect on growth	Mayer et al. (1977)
Salmo gairdneri (embryo, eyed egg)	10ºC Fl N	Acetone Conc.?	90 days	reduced vertebral collagen levels	LOEC NOEC	0.014 0.005	No effect on growth	

Table 3.72 continued overleaf

Table 3.72 continued Chronic (long term) toxicity to fish exposed via the water

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Pimephales promelas (juvenile 10 d)	25°C Fl N	Acetone Conc.?	127 days	reduced vertebral collagen levels increased hydroxyproline levels in collagen	LOEC NOEC	0.011 < 0.011	No effect on growth	

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations

Table 3.73 Fish: Embryo larval studies, short term and long-term exposure

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Ictalurus punctatus (Embryo larval)	29-31°C Se N	Acetone 0, 4 - 4 mg/l	7 days	Mortality 4 d post hatch	LC50 95% C.L NOEC	0.69 0.55-0.86	DOP, DEHP according to. Canadian Water Criteria Guidelines	Birge et al. (1978)
Lepomis microlupus (Embryo larval)	20-24 °C Se N	Acetone 0, 4 - 4 mg/l	7-8 days	Mortality 4 d post hatch	LC50 95% C.L NOEC	6.18 4.65 - 8.04		
Micropterus salmoides (Embryo larval)	20-24 °C F M		7-8 days	Mortality 4 d post hatch	LC50 NOEC LC50 NOEC	55.7 45.5	Hardness 45-55 mg CaCO3/l Hardness 190-225 mg CaCO3/l	Birge et al. (1979)
Micropterus salmoides (Embryo larval)	20-24 °C F M		7-8 days	Mortality 4 d post hatch	LC50 NOEC LC50 NOEC	42.1 32.9	Hardness 45-55 mg CaCO3/l Hardness 190-225 mg CaCO3/l	Birge et al. (1978) Same study as Birge et al 1979 but slightly different LC_{50}-values reported.

Table 3.73 continued overleaf

Table 3.73 continued Fish: Embryo larval studies, short term and long-term exposure

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Salmo gairdneri (Embryo larval)	12.5-14.5°C F M		26 days	Mortality 4 d post hatch	LC50 NOEC LC50 NOEC	139.5 149,2 	Hardness 45-55 mg CaCO3/l Hardness 190-225 mg CaCO3/l	Birge et al. (1978) Birge et al. (1979)
Carassius auratus (Embryo larval)	T? F M		8 days	Mortality 4 d post hatch	LC50 NOEC LC50 NOEC	> 186 > 191 	Hardness 45-55 mg CaCO3/l Hardness 190-225 mg CaCO3/l	Birge et al. (1979)
Salmo gairdneri (Embryo-larval)	10°C Fl M	Acetone 0.28 ml/l	102 days (12+90)	Hatchability mortality 5 d post hatch	NOEC NOEC LOEC	> 0.05 0.005 0.014		Mehrle and Mayer (1976)
Oncorhynchus mykiss (Embryo-larval)	10 ± 1°C Fl		90 days	Hatchability survival growth	NOEC	> 0.502		DeFoe et al. (1990)
Oncorhynchus mykiss (Embryo-larval)	Fl M		19 days		NOEC	> 0.00097	preliminary range finding study. embryos (approx. 24 h pre hatch) exposed until 18 days post hatch)	Rhodes and McAllister (1990)
Oncorhynchus mykiss (Embryo-larval)	Fl M		70 days	Hatchability survival growth	NOEC	> 0.0073		Cohle and Stratton (1992)
Jordanella floridae (Embryo-larval)	Se N		28 days	Mortality egg development growth	NOEC	>= 0.32	in Dutch	Adema et al. (1981)
Oryzias latipes (Embryo-larval)	Se N		28 days	Mortality egg development growth	NOEC	>= 0.32	in Dutch	

Table 3.73 continued overleaf

Table 3.73 continued Fish: Embryo larval studies, short term and long-term exposure

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Gasterosteus aculeatus	19±1°C Se		35 days	Mortality egg development growth	NOEC	> 0.32		Van den Dikkenberg (1989)
Brachydanio rerio (Embryo-larval)	Se N		35 days	Mortality development growth	NOEC	> =1	in Dutch	Canton et al. (1984)
Oryzias latipes (Embryo-larval)	Se N	Ethanol < 100µg/l	newly fertilised eggs exposed until hatch	Hatching time, hatching success, sex ratio, GSI, mortality, body weight			Test concentrations 0, 0.01, 0.1, 1.0 and 10.0 µg/l. No dose dependent effects were seen except for decreased body weights in male fish.	Chikae et al. (2004a)
Oryzias latipes (Embryo-larval)	Se N	Ethanol	3 weeks after hatching	mortality, body weight, sex ratio, GSI,			Test concentrations 0, 0.01, 0.1, 1.0 and 10.0 µg/l. Effects on body weight, GSI and mortality were observed., however, not dose dependent.	Chikae et al. (2004b)
Oryzias latipes (Embryo-larval)	S N	Acetone	5 days	Blood samples analysed for vitellogenin			Test concentrations 0, 10, 50 and 100 µg/l.	Kim et al. (2002)
Oryzias latipes (Embryo-larval)	Se N		1 or 2 dph to 3 months	Vitellogenin, GSI and histological analysis of reproductive organs			Test concentrations 0, 10 and 50 µg/l.	Kim et al. (2002)
Oryzias latipes (Embryo-larval)	Se N	Acetone	1 dph- 90 dph	Sex ratio, intersex morphometry			Test concentrations 0, 500, 1000 and 5000 µg/l.	Metcalfe et al, (2001)

Table 3.73 continued overleaf

Table 3.73 continued Fish: Embryo larval studies, short term and long-term exposure

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exposure period	Endpoint	Effect conc. (mg/l)		Comment	Reference
Oryzias latipes (Embryo-larval)	Se N	Acetone <100µg/l	Adult males exposed 2 weeks	No. of eggs, hatching rate			Test concentrations 39, 120 and 390µg/l.	Shioda and Wakabashi (2000)

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations

Table 3.74 Fish: Chronic (long term) toxicity to fish exposed via the diet (or intra-peritoneally)

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Exposure period	Test duration	Endpoint, effect parameter	Effect conc. (mg/kg in food)		Comment	Reference
Brachydanio rerio			90 days	Reproduction rate Fry survival	NOEC LOEC	< 50 50	Test conc,.50 and 100 mg/kg in food Considered invalid due to 49% mortality in control	Mayer and Sanders (1973)
Poecilia reticulata			90 days	Reproduction rate	NOEC	100	Test conc:100 mg/kg in food, Non-significant effects were observed	
Gadhus morhua			121 days	Steroid metabolism	NOEC LOEC	10 100	No significant differences in steroid metabolic profiles in male fish at highest dose (1000 µg DEHP/g food) compared to control. In female fish there was a significant alteration of steroid biosynthetic pathways in the head kidneys and ovaries of the DEHP-fed fish. The ratios of 11-deoxycortisol from 100 and 1000 µg DEHP/g groups were greater than twice the observed ratios obtained from the control and 10 µg DEHP/g.	Freeman et al. (1981)
Salmo salar		4 weeks	5 m	Sex ratio and liver somatic index	NOEC LOEC	300 1,500	Test conc.: 300 and 1500 mg/kg food	Norrgren et. al (1999)

Table 3.74 continued overleaf

Table 3.74 continued Fish: Chronic (long term) toxicity to fish exposed via the diet (or intra-peritoneally)

Species (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Exposure period	Test duration	Endpoint, effect parameter	Effect conc. (mg/kg in food)		Comment	Reference
Salmo salar		4 weeks	9 m	Intersex (ovotestis)	NOEC LOEC	800 1,500	Test conc.: 400, 800 and 500 mg/kg food	Norman et al (manuscript)
Salmo gairdnerii		7 weeks		Lipid metabolism	NOEC LOEC	< 20,000 20,000	Test conc.: 20g/kg	Henderson and Sargent (1983)

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations

Table 3.75 Chronic (long term) toxicity to Amphibians

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	Reference
	Exposure via water						**(mg/l)**		
1	*Xenopus laevis* (newly spawned eggs)	21°C Se	methyl alcohol (10ml/6L),?	3 m	larval development, growth, survival	see comment	-	Nominal concentrations: control, solvent control, 0.5, 1, 5, 20 mg/l. The test organisms were exposed from newly spawned eggs until fully developed frogs The results indicate an embryotoxic effect of DEHP, but there was no dose-response relationship The observed effects were also seen for the solvent control (methyl alcohol) No conclusion regarding effects of DEHP can be drawn from this study	Dumpert and Zietz (1983), Dumpert. (1981)

Table 3.75 continued overleaf

Table 3.75 continued Chronic (long term) toxicity to Amphibians

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	Reference
	Exposure via water						**(mg/l)**		
2	*Xenopus laevis* (newly spawned eggs)	Se M	DEHP in 1/5 Holfreter solution	200 d	retarded development time, reduced pigmentation of tadpoles	NOEC LOEC	< 2 2	Measured, the only concentration tested (single application and repeated weekly application), tap water control, Holfreter control. Effect at repeated application, no effect at single application of DEHP The test organisms were exposed from newly spawned eggs until fully developed frogs The developmental time was doubled at DEHP-exposure Developmental retardation, to a lesser degree, was also observed for the Holfreter control. A high frequency of unhatched eggs (> 50%) in all treatments as well as in the controls.	Dumpert and Zietz (1983), Dumpert. (1981)
3	*Bufo fowleri* (newly fertilised eggs)	20-24°C Se (12h) N?	acetone (0.04 4000 µg/l, depending on toxicant conc.)	7-8 d	survival	LC_{50} LC_1(extrapolated)	3.88 0.06	Nominal? (”Exposures were based on initial toxicant concentration”) control, acetone control, 0.01, 0.1, 1, 10, 100 mg/l. The chemical is named dioctylphthalate (DOP), but is actually DEHP according to the Canadian water criteria guidelines (1993). The test organisms were exposed from newly fertilised eggs to 4 days old tadpoles, Hatching occurred at days 3-4. The solvent was added at different concentrations, but no significant effect was observed in the solvent control. The study is considered invalid for the purpose of the risk assessment.	Birge et. al. (1978)
4	*Rana pipiens* (newly fertilised eggs)	20-24°C Se (12h) N?	acetone (0.04 4000 µg/l, depending on toxicant conc.)	7-8 d	survival	LC_{50} LC_1 (extrapolated)	4.44 0.18	Nominal? (”Exposures were based on initial toxicant concentration”) control, acetone control, 0.01, 0.1, 1, 10, 100 mg/l. The chemical is named dioctylphthalate (DOP), but is actually DEHP according to the Canadian water criteria guidelines 1993). The test organisms were exposed from newly fertilised eggs to 4 days old tadpoles, Hatching occurred at days 3-4. The solvent was added at different concentrations, but no significant effect was observed in the solvent control. The study is considered invalid for the purpose of the risk assessment.	Birge et. al. (1978)

Table 3.75 continued overleaf

Table 3.75 continued Chronic (long term) toxicity to Amphibians

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	Reference
	Exposure via water						**(mg/l)**		
5	*Rana arvalis* Moor frog (2-3 days old eggs)	5°C St M	ethanol (equal amounts in all treatments	60 d	Hatching success, tadpole survival rate	NOEC LOEC EC_{50}	25 75 650	Nominal concentrations, solvent control, 10, 25, 50, 200, 400, 800 mg/kg ww. Measured concentrations 0.9, 8.8, 23.7, 54.5, 119.5, 131.3, 431.4, 784.8 mg/kg ww The test organisms were exposed from 2-3 days old eggs to about 40 days old tadpoles. The tadpoles started to hatch after 3 weeks of exposure. Five replicates, 100 eggs per replicate. DEHP in ethanol (volume not known) added directly to wet sediment without evaporation of the vehicle before the exposure started. DEHP purity 97% The dose- response curve levels off. LOEC and NOEC were not reported. For this evaluation sample is read from the dose-response curve and LOEC is set as the first concentrations (measured) that significantly ($p < 0.05$) differs from the control according to one-tailed T-test. (The next lower dose =NOEC). A dry weight content of about 33% has been used to convert the concentrations to dw.	Larsson and Thurén (1987)
6	*Rana arvalis* Moor frog (4 days old eggs)	10°C St M	acetone	29 d	Hatching success, tadpole survival rate, growth	NOEC LOEC	433 >433	Nominal concentrations: Control, solvent control, spiking method control (ethanol, 300 mg/kg dw) 15, 30, 50, 100, 150, 300, 600 mg/kg dw. Measured conc.: 2.5, 2.5, 305, 13, 28, 41, 77, 137, 223, 433 mg/kg dw.. The tadpoles started to hatch after 1.5 weeks of exposure The test organisms were exposed from 4 days old eggs to about 20 days old tadpoles. DEHP added with acetone to dried sediment. Vehicle evaporated before start of exposure. DEHP purity 99% No statistics are reported. Standard errors for the means overlapped, why no further statistical analysis was conducted. No effect was observed for the ”Ethanol” control..	Wennberg et. al. (1997)

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations
5) References

Table 3.76 Acute (short term) toxicity to aquatic invertebrates

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	References
	Exposure via water					**mg/l**			
1	*Daphnia magna* (≤ 24 h)	20°C M	-	48 hours	Survival	1) EC_{50} NOEC 2) EC_{50} NOEC 3) EC_{50} EC_{100} 4) EC_{50} NOEC 5) EC_{50} NOEC 6) EC_{50} EC_{100}	> 0.113 0.113 > 0.102 0.102 < 1.03 < 1.03 > 0.114 0.114 > 0.166 0.166 < 1.04 < 1.04	6 test protocols. Concentrations in the tests: 1) 8.8, 18, 24, 35, 47, 71, 94, 113 µg/l 2) 8.0, 16, 21, 32, 42, 64, 85, 102 µg/l 3) 1.0, 5.1, 10,2 mg/l 4) 8.9, 18, 24, 36, 48, 72, 95, 114 µg/l 5) 13, 26, 35, 52, 69, 104, 138, 166 µg/l 6) 1.0, 5.2, 10.4 mg/l Tests without solubilizer. In four tests with concentration up to 0.17 mg/l no surface film or flotation of daphnids were observed. In these tests no adverse effects were observed at the highest tested concentration. In two tests with concentrations above 1 mg/l a surface film of DEHP and flotation of daphnids were reported. In these tests all daphnids died in all test concentrations.	Buchen and Vogel (1995)
2	*Daphnia pulex* (≤ 24 h)	17°C St N	Acetone, ≤ 0.5 ml/l	48 hours	Immobi-lisation	EC_{50}	0.133	5 concentrations and a solvent control. The report comprises acute toxicity test with *Daphnia pulex* on 30 compounds. The authors reported that, for some substances, the nominal concentration required to achieve 50% or greater immobilisation of organisms exceeded the solubility, as evidenced by cloudiness, precipitation or a surface film. Substances with solubility problems are noted in a table. However, DEHP has no such note.	Passino and Smith (1987)
3	*Daphnia magna* (≤ 24 h)	20°C St M	-	48 hours	Survival	LC_{50} NOEC	> 0.16 0.16	Mean measured concentration value from test performed with a control and a single test concentration at or near the water solubility limit of DEHP. No adverse effects observed at this concentration. DEHP was not acutely toxic up to its apparent limit of water solubility in the test solution. No solvent used.	Adams et al. (1995) Cox and Moran (1984) Springborn bionomics (1984a)

Table 3.76 continued overleaf

Table 3.76 continued Acute (short term) toxicity to aquatic invertebrates

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	References
	Exposure via water						**mg/l**		
4	*Daphnia magna* (≤ 24 h)	20°C St M	Acetone, 0.5 ml/l	48 hours	Immobi-lisation	EC_{50} NOEC	> 0.304 0.304	Nominal conc.: 0 (control), 0 (solvent control), 0.056, 0.10, 0.18, 0.32 mg/l. Mean measured conc.: 0 (contr.), 0 (solv. contr.), 0.047, 0.088, 0.169, 0.304 mg/l. No adverse effects were observed at the highest tested concentration. Daphnia floated on the surface after 48 hours, 25% at 0.169 mg/l and 100% at 0.304 mg/l.	Brown and Thompson (1982a)
5	*Daphnia carinata*	20°C	?	24 hours	Immobi-lisation	EC_{50}	0.33	Test concentrations not reported. The study comprised toxicity tests on many substances with seven organisms for comparison. Test performance and results are very briefly described. No floating Daphnia or surface film mentioned.	Yoshioka et al. (1987)
6	*Daphnia magna* (≤ 24 h)	20°C St N	Tween 20, 10 mg/l Marlowe t R 0, 10 mg/l	48 hours	Immobi-lisation	EC_{50} NOEC	> 1 1	Nominal concentrations: 0 (control), 0 (solubilizer control), 1 mg/l. Two tests with different dispersants. No immobilisation and no floaters at the tested concentration.	Brown et al. (1998)
7	*Daphnia magna* (≤ 18 h)	24°C St N	Ethanol, 1 ml/l	48 hours	Survival	EC_{50} NOEC LOEC	2.0 < 1 1	Concentrations: 0 (control), 0 (solvent control), 1, 2.5, 5, 10, 20 mg/l. One of the reports, Adams andHeidolph (1985), comprises acute toxicity tests with *Daphnia magna* on 18 substances. Adams (1978) appears to be the original report on the DEHP test. No floating Daphnia or surface film mentioned.	Adams and Heidolph (1985) Adams (1978)
8	*Daphnia magna* (≤ 24 h)	22°C St N	?	48 hours	Survival	LC_{50} NOEC	11 1.1	5 or more concentrations, a control and (probably) a solvent control. The report comprises acute toxicity test with *Daphnia magna* on 74 chemical substances. No floating Daphnia or surface film mentioned. For DEHP probably a co-solvent was used (triethylene glycol, ethanol, acetone or dimethylformamide) or the chemical was added directly to the diluent water.	LeBlanc (1980)
9	*Daphnia magna* (≤ 24 h)	20°C St N	Marlowe t R 40, 100mg/l	48 hours	Immobi-lisation	EC_{50} NOEC	> 100 100	Nominal concentrations: 0 (control), 0 (solubilizer control), 100 mg/l. The geometric means of measured concentrations at 0 hours and 48 hours do not deviate by more than 20% from the nominal values. The nominal values are used. No floating Daphnia or surface film mentioned No adverse effects observed at this concentration	Scholz (1995a)

Table 3.76 continued overleaf

Table 3.76 continued Acute (short term) toxicity to aquatic invertebrates

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	References
	Exposure via water						**mg/l**		
10	*Moina macrocopa* (≈ 5 d)	20°C St	-	3 hours	Survival	LC_{50}	-	Test concentrations not reported. The study comprised toxicity tests on 22 substances with four organisms for comparison. The LC_{50} is reported to be higher than the highest tested concentration (≥ saturation).	Yoshioka et al. (1986)
11	*Nitocra spinipes* (Adult) Br	21°C St N	Acetone ≤ 0.5 ml/l	96 hours	Survival	LC_{50}	> 300	6 or more concentrations, a control and (probably) a solvent control. Test performed in brackish water, salinity 7%. The report comprises acute toxicity test with *Nitocra spinipes* on 78 compounds.	Lindén et al. (1979)
12	*Gammarus pulex* (Two groups: >8 mm and <5 mm)	7and 15°C Se N	Acetone, ≤ 0.66 ml/l	96 hours	Survival	LC_{50} NOEC	> 0.4 0.4	Solvent control. In hard water 5 concentrations; 1-10 mg/l. In soft water 2 concentrations 5 and 10 mg/l. The study was designed to examine the effects of water hardness, water temperature, size of test organism, and duration of exposure on the acute toxicity of four substances, including DEHP, to *G. pulex*. After 96 hours exposure to the test substance the test animals were returned to toxicant-free water for 24 hours." DEHP was not acutely lethal in any of the experiments, despite the maximum concentrations in all tests being in excess of its solubility in water, 0.4 mg/l".	Stephenson (1983) Stephenson (1982)
13	*Gammarus pseudolimnaeus*	St N	-	96 hours	Survival	LC_{50}	> 10	Test concentrations not reported. The test performance and results are very briefly described. The text indicates solvent not to be used.	Mayer and Sanders (1973)
14	*Gammarus pseudolimnaeus*	21°C St ?	Triton X-100	96 hours	Survival	LC_{50}	> 32	Test concentrations not reported. The test performance and results are very briefly described.	Sanders et al. (1973) Johnson and Finley (1980)
15	*Mysidopsis bahia* (≤ 24 h) Ma	20°C St M	-	96 hours	Survival	LC_{50} NOEC	> 0.37 0.37	Mean measured concentration value from test performed with a control and a single test concentration at or near the water solubility limit of DEHP. No adverse effects observed at this concentration. DEHP was not acutely toxic up to its apparent limit of water solubility in the test solution. No solvent used. The salinity in the test water was 22%.	Adams et al. (1995) Cox and Moran (1984)

Table 3.76 continued overleaf

Table 3.76 continued Acute (short term) toxicity to aquatic invertebrates

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	References
	Exposure via water						**mg/l**		
16	*Orconectes nais*	St N	-	96 hours	Survival	LC_{50}	> 10	Test concentrations not reported. The test performance and results are very briefly described. The text indicates solvent not to be used.	Mayer and Sanders (1973)
17	*Paratanytarsus parthenogenetica* (larvae, 2nd or 3rd instar)	20°C St M	-	48 hours	Immobilisation	EC_{50} NOEC	> 0.18 0.18	Mean measured concentration value from test performed with a control and a single test concentration at or near the water solubility limit of DEHP. No adverse effects observed at this concentration. DEHP was not acutely toxic up to its apparent limit of water solubility in the test solution. No solvent used. Benthic organism.	Adams et al. (1995) Cox and Moran (1984)
18	*Paratanytarsus parthenogenetica* (larvae, 3rd or 4th instar)	23°C St N	dimethyl - formamide, ≤ 0.5 ml/l	48 hours	Survival	LC_{50} NOEC LOEC	16.3 1.25 2.5	Concentrations: 0 (control), 0 (solvent control), 0.62, 1.25, 2.5, 5.0, 10 mg/l. The LC_{50} was higher than the highest tested concentration (10 mg/l) and was estimated by the probit method to be 16.3 mg/l. Benthic organism.	Adams and Renaudette (1983)
19	*Chironomus tentans* (larvae)	Fl M?	?	10 days	survival?	LC_{50} NOEC	> 0.046 0.046	No adverse effects were observed at the highest tested concentration Benthic organism. The final report is not yet available	CMA, 1997
20	*Chironomus tentans* (larvae, 2nd instar)	21°C St N	Yes, but not specified	48 hours	Survival	LC_{50} NOEC	> 10 10	Concentrations: 0 (control), 0 (solvent control), 10 mg/l. No adverse effects were observed at the highest tested concentration, which exceeded the solubility in the test solution. Benthic organism	Adams and Calvert (1983)
21	*Chironomus plumosus* (larvae, 2nd, or 3rd-4th instar)	St N?	ethanol< 1.8 ml/L	48 hours	Immobilis. Mortality: 2nd inst. 3rd-4th inst.	EC_{50} LC_{50} LC_{50}	> 18 > 18 > 18	The concentration range was not reported Benthic organism.	Streufert (1977) Streufert et al. 1980

Table 3.76 continued overleaf

Table 3.76 continued Acute (short term) toxicity to aquatic invertebrates

Study No5	Species, organism (Life stage, age) Ma, Br1	T2 St, Se, Fl3 M, N4	Vehicle	Exp. period	End-point	Effect conc.		Comment	References
	Exposure via water						**mg/l**		
22	*Hyalella azteca*	Fl M?	?	10 days	survival?	LC_{50} NOEC	> 0.057 0.057	No adverse effects were observed at the highest tested concentration Benthic organism. The final report is not yet available	CMA, 1997
23	*Dugesia japonica* flatworm (≈ 2cm)	20 Se	-	7 days	head regene-ration	EC_{50}	-	Test concentrations not reported. The study comprised toxicity tests on 22 substances with four organisms for comparison. The flatworms were cut into two parts, head and body. The body part was used for the test and observed for abnormal head regeneration. The LC_{50} is reported to be higher than the highest tested concentration (≥ saturation).	Yoshioka et al. (1986)
	Exposure via sediment						**mg/kg dw**		
24	*Chironomus tentans* (larvae)	Fl	?	10 days	survival	LC_{50} NOEC	>3,247 3,247	Only one concentration tested?	CMA, 1997
25	*Hyalella azteca*	Fl	?	10 days	survival	LC_{50} NOEC	>3,306 3,306	Only one concentration tested?	CMA, 1997

1) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in ºC
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations
5) References

Table 3.77 Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
1	*Daphnia magna* (≤ 24 h)	21°C Se N?	Ethyl alcohol, 0.1 ml/l	21 days	Reproduction	NOEC LOEC EC60	< 0.003 0.003 0.003	Concentrations: solvent control, 3, 10, 30 µg/l Food: yeast suspension. Low reproduction rate (11 offspring per adult), which is lower than the condition for validity in the OECD guideline 202. The USEPA has excluded the result from the data used for Ambient Water Quality Criteria. The test result is considered invalid for the purpose of this risk assessment.
2	*Daphnia magna* (≤ 24 h)	22°C Fl M	Acetone, < 50µl/l	21 days	DNA-content, RNA/DNA-ratio at day 7 Survival, Reproduction	NOEC LOEC NOEC LOEC	0.072 0.158 0.158 0.811	Nominal concentrations: 0, 75, 150, 300, 600, 1,200 µg/l. Measured concentrations: 0, 12, 27, 72, 158, 811 µg/l. Food: *Selenastrum capricornutum*, yeast suspension and PR11 trout chow. Reproduction rate: 200 young per adult. The study was conducted at the same facility as used by Sanders et al (1973). Daphnids were trapped at the surface (dose and time dependent) but appeared to be feeding and healthy. Regarding surfacing, lowest concentration significantly different from control was 158 µg/l day 0. As they grew, the number at the surface decreased. By day 21 surfacing behaviour was observed only in daphnids exposed to 811 µg/l. Static biochemical indicators, e.g. content of protein, RNA, DNA and glycogen, have been shown to be closely associated with total growth (e.g. larger Daphnia contain more protein and DNA). Dynamic biochemical indicators as Protein/RNA and RNA/DNA ratios have been shown to reflect growth rate.The reduced survival and reproduction of Daphnia exposed to 811 µg/l for 21 days were preceded by reduced levels of all biochemicals measured at day 7, except total lipids.

Table 3.77 continued overleaf

Table 3.77 continued Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
3	*Daphnia magna* (≤ 24 h)	21°C Fl M	-	21 days	Survival Reproduction	NOEC LOEC NOEC LOEC	0.077 0.16 0.29 > 0.29	Measured concentrations: 0, 26, 45, 77, 160, 290 µg/l Food : Unicellular algae, *Ankistrodesmus sp*. and yeast suspension. No co solvent. A gas-tight syringe with a mechanical injector was used to introduce a desired amount directly into the chemical mixing chamber. Ultrasonic dispersion in the chemical mixing chamber. The report comprises chronic toxicity test with *Daphnia magna* on 14 phthalates. Reproduction rate, mean offspring per adult female in the 14 tests, ranged from 56 to 116. In the DEHP-test; 56. At 0.29 mg/l daphnids were entrapped on the surface throughout the study. At 0.16 mg/l daphnids were entrapped on the surface day 7 and 14, not day 21. At 0.077 mg/l daphnids were entrapped on the surface day 7, not day 14 and 21. In the control daphnids were entrapped on the surface throughout the study. This entrapment did not affect the daphnids survival or reproduction.
4	*Daphnia magna* (≤ 24 h)	20°C Se N	Acetone, 0.5 ml/l	21 days	Survival, Reproduction	NOEC LOEC	0.1 > 0.1	Nominal concentrations: 0 (control), 0 (solvent control), 3.2, 10, 32, 100 µg/l. Mean Measured concentrations, "new-old": 0, 3.08-1.33, 10.4-4.3, 33.6-17, 107-64.3 µg/l. Food : *Chlorella vulgaris* and yeast suspension Reproduction rate approximately 170 young per adult. No effect at highest tested concentration, 0.1 mg/l. The parent Daphnia showed bioconcentration factors of 209. In an acute toxicity test Daphnia floated on the surface after 48 hours, 25% at 0.169 mg/l and 100% at 0.304 mg/l. In the reproduction test (≤ 0.1 mg/l) no floating Daphnia was reported. The authors also stated that a solubility/stability-test indicated that at levels below 0.180 mg/l DEHP gives stable solutions, whereas above this level loss of phthalate from the bulk solution occurs.
5	*Daphnia magna* (≤ 24 h)	21-23°C Se M	dimethyl-formamide ≤ 0.1 ml/l	21 days	Survival Reproduction Growth (7d)	NOEC LOEC NOEC LOEC NOEC LOEC	0.640 1.300 0.640 1.300 1.30 > 1.30	5 or more concentrations, a control and a solvent control. Food: PR11 trout chow Reproduction rate unknown. No floating Daphnia or surface film mentioned. The report comprises chronic toxicity test with *Daphnia magna* on 20 substances.

Table 3.77 continued overleaf

Table 3.77 continued Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
6	*Daphnia magna* (≤ 24 h)	20°C Se N	Marlowet R 40, 10 mg/l	21 days	Survival Reproduction Growth	NOEC LOEC	1.0 > 1.0	Nominal concentrations: 0 (control), 0 (dispersant control), 1.0 mg/l. Food: *Chlorella vulgaris* and commercially available microincapsulated food "Frippak Booster". The reproduction rate in the controls ranged from 120 to 131 mean offspring per adult. No adverse effects at highest tested concentration, 1 mg/l. No floaters were observed.
7	*Daphnia magna* (≤ 24 h)	20°C Se N	Tween 20, 2.5 mg/l at test conc. 0.25 mg/l, 10 mg/l at test conc. 1 mg/l	21 days	Survival Reproduction Growth	-	See comment	Nominal concentrations: 0 (control), 0 (dispersant control 10 mg/l), 0.25, 1.0 mg/l. Food: *Chlorella vulgaris* and commercially available microincapsulated food "Frippak Booster". The reproduction rate in the controls ranged from 155 to 167 mean offspring per adult. At both concentrations there was a reduction in numbers of offspring produced relative to the dispersant control. The reduction was significant at 0.25 mg/l but not at 1 mg/l. Relative to the control without dispersant the reduction in numbers of offspring was not significant in any of the concentrations. There were no significant mortalities in the test. At both concentrations there was a reduction in length produced relative to the control without dispersant. The reduction was significant at 0.25 mg/l but not at 1 mg/l. Relative to the dispersant control the reduction in length was not significant in any of the concentrations. No floaters were observed.
8	*Daphnia magna* (≤ 24 h)	20°C Se M	Marlowet R 40, 30 mg/l	21 days	Survival, Reproduction	NOEC LOEC	14 > 14	Nominal concentrations: 0 (control), 0 (solubilizer control), 0.8, 2.0, 5.0, 12, 30 mg/l. Mean Measured concentrations, "freshly prepared": 0.31, 0.90, 2.2, 5.3, 14 mg/l. Food : *Scenedesmus subspicatus* Reproduction rate approximately 70 young per adult in control and 100 in test concentrations. No adverse effects at highest tested concentration, 14 mg/l. The reproduction rate was significantly higher in all test concentrations and in the solubilizer control compared to controls. It is concluded in the report that "it is evident from the data that Marlowet R 40, the solubilizer employed, slightly increased the number of offspring during the study." No floaters reported.

Table 3.77 continued overleaf

Table 3.77 continued Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
9	*Gammarus pulex* (>12 mm)	10-12°C Fl N	Ethanol, <0.025 ml/l	10 days	Mortality, Locomotor activity	NOEC LOEC NOEC LOEC	0.5 > 0.5 0.1 0.5	Nominal concentrations: : 0 (solvent control), 0.1, 0.5 mg/l. The 25 day test was divided into pre-exposure (5 days), exposure (20 days), and post-exposure (10 days). The authors commented that the DEHP adsorbed to and accumulated by the organisms could have caused organs sensitive to water velocity, and "olfactory" organs to malfunction thereby reducing the mobility and affecting upstream movement. The decreasing activity following DEHP exposure could therefore have been the result of mechanical and/or physiological effects. Accumulated in organisms 2,000 mg/kg at 0.5 mg/l exposure (BCF 4000). Adsorbed to the organisms integument 2,300 mg/kg. The bottom substrate consisted of sand and decaying alder leaves and patches of pebbles.
10	*Chironomus plumosus* (larvae, 1st instar)	Fl M/N	ethanol <0.12 ml/L	35-40 days	midge emergence, production and hatchability of eggs	Sand NOEC LOEC hydros. NOEC LOEC	 0.36 > 0.36 0.24 > 0.24	Test was carried out in two different systems, one with sand and one with hydrosoil present on the bottom of the experimental systems. The exposure concentration in water was measured in the system with the highest exposure level once during the experiment and a correction factor was determined from this measured value relative to the nominal conc. Sand reduced the concentration of DEHP in solution by 28% while hydrosoil reduced the concentration by 62% . All nominal exposure concentrations were corrected with those correction factors. Mean concentrations obtained in the sand systems were: 0, 0.14, 0.20, 0.36 mg/l Mean concentrations obtained in the hydrosoil systems were: 0, 0.11, 0.20, 0.24 mg/l. The bioaccumulation factors after 8 days for animals exposed to 267 ng DEHP/L was 573 in the presence of sand and 738 in the presence of hydrosoil. Benthic organism.
11	*Palaemonetes pugio* grass shrimp (larvae <24 h) Ma	22°C Se N	Acetone, ≤ 1 ml/l	28 days	Survival, Molting rate, Duration of zoeal development	NOEC LOEC	1.0 > 1.0	Nominal concentrations: 0 (solvent control), 0.1, 0.5, 0.75, 1.0 mg/l. Mean Measured concentrations: "new-old" 0.097-0.027, 0.368-0.138, 0.529-0.301, 0.510-0.39 mg/l. Artificial seawater with salinity of 17%. Initial experiments to determine the solubility in the test medium (artificial sea water) indicate DEHP to be soluble to not more than 1 ppm. At the highest concentration, small droplets of DEHP were sometimes observed indicating incomplete equilibrium.

Table 3.77 continued overleaf

Table 3.77 continued Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water					**mg/l**		
12	*Mytilus edulis* (Mean shell length 22.6 mm, range 20-28. Mean wet tissue weight 472 mg, range 274-772) Ma	15°C Fl N	Acetone, 0.5 ml/l	28 days	Deposition of faecal/pseudo-faecal material, Byssal thread attachment, General appearance, Activity, Survival	NOEC LOEC	0.05 > 0.05	Bioconcentration study that also have been referred to as a toxicity test. Exposure 28 days, depuration period 14 days. ^{14}C labelled DEHP. Nominal concentrations: 0 (solvent control), 0.005, 0.05 mg/l. Mean Measured concentrations: 0.0041, 0.0421 mg/l. Bioconcentration reaches plateau level at around day 14. BCF 2500 at plateau level. No adverse effects at highest tested concentration, 0.05 mg/l.
	Exposure via sediment					**mg/kg dw**		
13	*Chironomus riparius* (larvae, 1st instar)	20°C St M	acetone	28 days	delayed emergence, number of emerged adults	NOEC LOEC	11,000 > 11,000	DEHP concentrations in test sediments: 130, 1,200 and 11,000 mg/kg dw. The test substance was mixed with trace amounts of corresponding radiolabelled material and the concentrations in the sediments were analysed by radiochemistry. DEHP added with acetone to dried sediment. Vehicle evaporated before start of exposure The larvae were fed daily with uncontaminated food

Table 3.77 continued overleaf

Table 3.77 continued Chronic (long term) toxicity to aquatic invertebrates

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via sediment					**mg/kg dw**		
14	*Aeshna sp* Dragonfly (larvae)	22°C St M	ethanol	60 days	predation efficiency	NOEC LOEC	780 1,560	One solvent control and two DEHP contaminated systems at 587 and 623 mg/kg ww. (Average 600 mg/kg ww). DEHP in ethanol added directly to wet sediment (50ml/ 5L). The sediment was left for five days to equilibrate, before water and test organisms were added. Measured conc. in control sediment was 1 mg/kg dw. Experimental larvae were collected from a natural lake, acclimatised (3 w) in test aquaria (20 larvae per aquaria). Predation efficiency was then studied for 40 days. "A significant effect" (15-25% reduction in predation efficiency) was observed in the DEHP aquaria, compared to controls. NOEC set to LOEC/2,. A factor of 2.6 was in this RA used to convert ww concentrations to dw.

1) Marine/ Brackish organism (otherwise freshwater)
3) Static, Semistatic or Flow through
2) Temperature in ºC
4) Measured or Nominal concentrations
5) References:
1) Mayer and Sanders (1973); Sanders et al (1973)
2) Knowles et al (1987)
3) Rhodes et al. (1995); Springborn bionomics (1984c);Cox and Moran (1984)
4) Brown and Thompson (1982a)
5) Adams and Heidolph (1985)
6) Brown *et. al*. (1998)
7) Brown *et. al*. (1998)
8) Scholz (1995b); Scholz (1994)
9) Thurén and Woin (1991)
10) Streufert (1977); Streufert *et. al*. (1980)
11) Laughlin *et. al*. (1978)
12) Brown and Thompson (1982b)
13) Thompson *et. al*. (1995); Brown et al. (1996)
14) Woin and Larsson (1987)

Table 3.78 Toxicity to algae and higher plants

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Algae						**Mg/l**	
1	*Scenedesmus quadricauda*	27°C St N	?	7 days	growth inhibition, NOEC=3 % threshold value	NOEC	10	Four parallel dilution series, each of the dilutions containing 1 part v/v of the pollutant solution in 2^0 to 2^{14} parts v/v "mixture". Initial concentration not reported but probably 400 mg/l according to the greater than LOEC value reported for *Pseudomonas putida* in the same study. Cell multiplication test. No vehicle was mentioned
2	*Scenedesmus subspicatus*	24°C St M	MARLOW-ET R 40, (100 mg/l),	72 hours	inhibition of cell growth and growth rate	NOEC LOEC	130 > 130	Control, solubilizer control, 130 mg/l. The test concentration was measured in separate vessels without algae. The test is performed according to 92/69/EEC, GLP DEHP = VESTINOL AH
3	*Ankistrodesmus bibraianus*	23°C St N	?	72 hours	growth inhibition	NOEC LOEC	0.0056 > 0.0056	Nominal concentrations: control and 0.007mg/l. The test protocol is unclear regarding the actual DEHP concentrations in tests and controls. The reported no effect concentration was measured in separate vessels without algae (?). The measured concentrations in the controls were almost the same as the concentrations in the test vessels in some replicates (?). Measured DEHP concentrations in the controls were 0.0045- 0.0048 (?) mg/l. The concentrations in tests and controls were close to the detection limit. The test is considered invalid.
4	*Selenastrum capricornutum*	22-24°C St M	-	96 hours	growth inhibition	NOEC EC_{50}	0.1 > 0.1	One single measured concentration No vehicle was used; The DEHP was injected directly into the test water and homogenised for 2 min with a Polytron blender. US EPA algal assay bottle test
5	*Gymnodinium breve* Ma	25°C St N	-	96 hours	growth inhibition	EC_{50}	30,000 (3.1%)	DEHP added directly into test vessel (containing artificial sea-water medium) at nominal volume percentages: 0, 0.1, 0.2, 0.5, 1, 2, 5, and 10.
	Macrophytes						**mg/l**	
6	*Lemna gibba*	25°C Se N?	?	7 days	growth inhibition	EC_{50}	397-7,582 (mean 2,060)	7 test series were carried out resulting in 7 EC_{50} estimates. The concentration ranges in the tests were not reported (only the dilution series, not the concentration of the stock solution). No vehicle mentioned. "The toxicant formed oil droplets or globules, or completely covered the surface of the test chambers, depending on the concentration.

1) **Ma**rine/ **Br**ackish organism (otherwise freshwater)
2) **T**emperature in ºC
3) **St**atic, **Se**mistatic or **Fl**ow through
4) **M**easured or **N**ominal concentrations
5) References:
   1) Bringmann and Kühn, 1980
   2) Hüls Aktiengesellschaft, 1995
   3) BASF AG, 1990
   4) Adams *et. al.,* 1995
   5) Wilson *et. al.,* 1978
   6) Davis, 1981

Table 3.79 Toxicity to micro-organisms

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
1	Natural pelagic community Ma	Outdoor temp. Se M	acetone	30 days	Reduction in NH_3 flux	NOEC winter NOEC summer	0.059 0.016	Marine microcosm, The test was run during summer as well as winter conditions ^{14}C-DEHP was added to the water at nominal concentrations of 1, 10 and 100 µg/l. 5 replicates per concentration. Approximately one tenth of the water was replaced three times per week and DEHP was added in quantities sufficient to achieve the original test concentration in the replacement water. The measured concentrations at the end of the experiment were for the winter situation 0.58, 5.8 and 59 µg/l respectively, and for the summer situation 0.18, 1.2, and 16µg/l.
2	*Pseudomonas putida*	St N?	?	16 hours	growth inhibition,	Threshold conc. = 3% effect	> 400	Four parallel dilution series, each of the dilutions containing 1 part v/v of the pollutant solution in 2^0 to 2^{14} parts v/v "mixture". Initial concentration not reported but probably 400 mg/l according to the greater than LOEC value., Vehicle not mentioned, Cell multiplication inhibition test,
3	*Pseudomonas putida*	22°C St N	TWEEN 80 (100mg/lm g/l)	30 m	respiration inhibition	-	-	According to DIN 38412/27 oxygen cons. test. Nominal concentrations: control, solvent control, 19, 39, 78, 156, 313, 625, 1,250, 2,500, 5,000, 10,000 mg DEHP/L. No dose-response effect: 20% inhibition at 19.5 mg/l (lowest conc.)-2,500 mg/l, no effect at higher conc., no effect at 5,000 and 10,000 mg/l. Therefore A NOEC cannot be derived. The study was considered invalid for the purpose for the risk assessment
4	*Pseudomonas putida*	25°C	nonylphen olethoxypr o-poxylat (1,5 ml/L)	5-6 hours	respiration inhibition	NOEC EC_{10}	< 1,671 1,671	Only one concentration tested 1.7 ml/L A density of 0.983 g/cm^3 was used to express the dose as mg/l (DEHP=VESTINOL AH)
5	*Uronema parduczi* protozoa	20°C St N?	?	20 hours	growth inhibition, NOEC=3 % threshold value	NOEC	48	The concentration range was not reported Vehicle not mentioned Cell multiplication inhibition test 160 potential pollutants were tested

Table 3.79 continued overleaf

Table 3.79 continued Toxicity to micro-organisms

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Sediment					**mg/kg dw**		
7	*Chilomonas paramecium Ehrenberg* protozoa	20°C St N?	?	48 hours	growth inhibition,	Threshold conc. = 5% effect	53	The concentration range was not reported Vehicle not mentioned. Cell multiplication inhibition test. 160 potential pollutants were tested saprozoic
8	Natural sediment flora	5°C St N	ethanol	20 hours (60) see comm.	Inhibition of oxygen consumption in overlaying water of sediment cores	NOEC= LOEC/2 LOEC=EC_{17})	43 87	Measured test concentrations: solvent control, 33, 57, 76, 200, 315 mg/kg ww. The oxygen consumption was recorded every 10 hours up to 60 hours. The water was resaturated after one week and oxygen consumption was studied for another 60 hours. The results are reported as curves for oxygen decrease over time, for each concentration. A dw correction factor of 2.6 is used. For this evaluation, the ECx for each concentration at 20 hours are read from the curves. The sediment concentrations were measured on homogenised sediment from the uppermost 5 cm of the cores, but the DEHP was initially added 5 mm below the sediment surface. Therefore concentrations in the surface layer were probably much higher. However, a much stronger effect was shown for the lower exposure concentrations when the water was resaturated after 7 days and the oxygen demand measured again. By this time the DEHP in the sediment should have had more time for levelling out the concentration by diffusive transport
9	Freshwater hydrosoil flora	Se	?	?	growth and suppression in physiological activity	NOEC LOEC	100mg/l > 100mg/l	Hydrosoil microcosm DEHP added to hydrosoil at nominal concentrations: 1 and 100 mg/l Suppression in physiological activity: nitrification, ammonification , sulphur reduction etc. The study is only available as a short abstract

Table 3.79 continued overleaf

Table 3.79 continued Toxicity to micro-organisms

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Sediment					**mg/kg dw**		
10	Natural benthic communityMa	outdoor temp. Se M	acetone	30 days	Reduction in NH_3 flux from the benthic compartment	NOEC LOEC	0.61 18.64	Marine microcosm, test was run during summer as well as winter conditions ^{14}C-DEHP was added to the water at nominal concentrations of 1, 10 and 100 µg/l, 5 replicates. One tenth of the water replaced three times per week and DEHP was added in quantities sufficient to achieve the original test concentration in the replacement water. DEHP accumulation in 0-7 cm of the sediment was measured after 30 days. NOEC and LOEC has been calculated by us from the 0-7 cm sediment value (reported per dw), corrected for 20% dw (standard in TGD) and an assumption by the authors that the actual exposure concentrations (that caused 30% reduced NH_3 flux) was that of the uppermost 3 cm, which was three times higher than the concentration in the 0-7 cm layer. Not clear if the measured NH_3flux was from a pure microbial benthic community or if macrobenthos were present. The variation between replicates is not reported.
	Activated sludge						**mg/l**	
11	activated sludge			24 hours	toxicity threshold	NOEC LOEC	< 10-20	from the IUCLID database, ETAD fermentation tube test, Literature not available activated sludge from a predominantly domestic sewage
12	digester sludge from municipal wastewater treatment plant	37°C St N	-	26 days	suppression of methanoge ne-sis	NOEC LOEC	100 200	Anaerobic toxicity assay Nominal: 20, 100, 200 mg/l tested, DEHP added directly to the test vessels
13	activated sludge	24-26°C St N	-	30 m	respiration inhibition	NOEC LOEC	< 0.4 0.4	Nominal: The only concentration tested (corresponding to the solubility limit according to the authors). Replicate tests. No statistics reported. The oxygen consumption was 86% of the control => EC14 Modified OECD method 209. 33 compounds tested

Table 3.79 continued overleaf

Table 3.79 continued Toxicity to micro-organisms

Study No[5]	Species, organism (Life stage, age) Ma, Br[1]	T[2] St, Se, Fl[3] M, N[4]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Activated sludge						**mg/l**	
14	activated sludge from BASF treatment plant	20°C St N	-	24 hours see comm.:	respiration inhibition	NOEC LOEC	1,960 (2 ml/L) > 1,966	Nominal concentrations: 0, 0.06, 0.1, 0.2, 1.0, 2.0 ml/L DEHP tested. a density of 0.983 g/cm^3 was used to express the dose as mg/l . The microbial population might have been adapted, as the inoculum originated from the BASF treatment plant Activated Sludge Respiration Inhibition Test (DEHP = PALATINOL AH) according to IUCLID was the exposure time 30 min
15	activated sludge from the municipal sewage treatment plant in Marl-West	18-21.1°C St N		3 hours	respiration inhibition	NOEC LOEC	2,007 > 2,007	The test was carried out according to OECD 209. Test concentrations 93, 236, 556, 1,039 and 2,007. The difference in respiration rate between the two controls was 1.9%. DCP was used as reference substance and the obtained EC_{50} was within the limits set in the guidance document.
	activated sludge from the municipal sewage treatment plant in Marl-West	19-20°C St N	Tween 80	3 hours	respiration inhibition	NOEC LOEC	1,000 > 1,000	The test was carried out according to OECD 209. Test concentrations 62, 125, 250, 500 and 1,000. The difference in respiration rate between the two controls was 1.0%. DCP was used as reference substance and the obtained EC_{50} was within the limits set in the guidance document.

1) **Ma**rine/ **Br**ackish organism (otherwise freshwater)
2) **T**emperature in ºC
3) **St**atic, **Se**mistatic or **Fl**ow through
4) **M**easured or **N**ominal concentrations
5) References:
1) Perez *et. al.,* 1983
2) Bringmann and Kühn, 1980.
3) BASF AG, 1991
4) Hüls AG, 1996
5) Bringmann and Kühn, 1981
6) Bringmann and Kühn, 1980; Bringmann and Kühn, 1981
7) Bringmann and Kühn, 1981
8) Larsson *et. al.,* 1986
9) Mutz and Jones, 19
10) Perez *et. al.,* 1983
11) -
12) O'Connor *et. al.,* 1989
13) Volskay and Leslie Grady, 1988
14) BASF AG, 1983
15) Hüls Infracor, 1999

3.2.2 Atmosphere

There is no information available concerning the impact of DEHP on atmospheric processes like global warming, ozone depletion in the stratosphere, ozone formation in the troposphere, and acidification etc. Given the physico chemical and exposure-related properties of DEHP it does not seem likely that it will have adverse effects on atmospheric processes like those listed above.

DEHP in the atmosphere is sooner or later deposited on the soil or surface water, as wet or dry deposition. Plants may take up a substance from the air directly through the stomata or may adsorb the substance on the surface of foliage and stems. Substances taken up in the plants may then be transported to soil via the roots or the defoliation. No information is available on this transport route for DEHP.

Methods for the determination of effects of chemicals on species arising from atmospheric contamination have not yet been fully developed, except for inhalation studies with mammals (TGD).

3.2.2.1 Direct exposure from the atmosphere

A Danish research group has exposed plants to DEHP in the air. In a field experiment they investigated the effects of the two phthalates DEHP and DBP, when applicated by spraying on the foliage of *Sinapis alba* and *Brassica napus* (Løkke and Rasmussen, 1983). No effect was observed at the maximum dosage of 8.75 µg/cm^2 of DEHP while chlorotic spots were observed on leaves treated with 2.19 µg/cm^2 of DBP or mixtures of DBP and DEHP (2.19 + 2.19µg/cm^2) (Løkke and Rasmussen, 1983). The authors concluded that DEHP seemed to have a very low mobility in plants. In another set of experiments the same research group used closed climate chamber to expose *Sinapis alba* to a mixture of DEHP, DBP and DiBP at foliage concentrations of about 2.5 µg/cm^2 of each phthalate (Løkke and Bro-Rasmussen, 1981). Chlorosis was observed but the authors concluded from additional experiments with DBP, that this effect was caused by DBP only. It was demonstrated in both these studies that the DEHP did not evaporate from the leaves. The authors also added droplets of pure DEHP, without solvents, on foliage. No adverse visible effect was observed from this treatment.

Hardwick et al. (1984) grew cabbage seedlings in a cuvette bioassay in the presence of strips of plastic plasticised with DBP, DEHP or DIDP. Effects were observed with plastics treated with DBP. No effects were observed with small samples of DIDP-plasticised strips. Effects were observed with larger samples of DIDP-plasticised strips, but residual concentrations of DBP were measured in the air while no DIDP was detected (limit of determination not indicated. No effects (and no DEHP in the headspace air) were observed with DEHP-plasticised strips at 30°C. The authors remarked that DINP and DEHP might be released and proved toxic at higher temperatures, referring to an old study by Inden and Tachibana (1975): “whilst the plasticisers DEHP and DIDP caused little or no damage to cucumber and Chinese cabbage seedlings at normal temperatures they could be induced to volatilise on being heated to 100-200°C and the air stream containing the vapours did then cause extensive damage to these plants.”

Al-Badry and Knowles (1980) applied DEHP topically, on the venter of abdomen, or by intrathoracic injection to female houseflies (*Musca domestica*). Mortality was checked after 24 hours of exposure to 20 µg DEHP in acetone (1µl when added topically and in 10% solution when injected), which was equivalent to 1,000 mg DEHP/kg bw. DEHP at this concentration did not have any observed effect on the flies when added alone. When applied simultaneously with

various organophosphates, an antagonistic interaction was apparent. However, when DEHP was applied 30 minutes before exposure to an organophosphate, the resulting interaction was synergistic, i.e. DEHP increased the lethal effect of the organophosphate.

3.2.2.2 PNEC atmosphere

From the studies above, no $PNEC_{atmosphere}$ can be derived in order to compare with $PEC_{atmosphere}$, calculated per cubic metre. However, a comparison can be made between the NOECs above and known deposition rates. Supposing that the housefly has an area of 1 cm^2, then a single dose of 20 µg DEHP would correspond to an isolated deposition occasion of 200 mg DEHP/m^2 (NOEC). The corresponding deposition value, calculated from the NOEC (8.75 µg/cm^2) for *Sinapis alba* and *Brassica napus,* would be 87.5 mg/m^2.

3.2.3 Terrestrial compartment

The terrestrial ecosystem comprises of an above-ground community, a soil community and a groundwater community. According to TGD it is so far not possible to carry out effect assessment for the groundwater community, since no toxicity tests/data exist for this compartment.

3.2.3.1 Toxicity to plants

The effect studies on plants are summarised in **Table 3.80** and **Table 3.81**.

There are only two studies with plants exposed to DEHP in soil. Herring and Bering (1988) found no effects on seedlings of *Spinacia oleracea* and *Pisum sativum* exposed to DEHP in soil, at the highest concentration. It is however unclear from the paper what the actual soil concentration was in the experiment and these results can therefore not be used in the risk assessment.

Diefenbach (1998a) studied the effects of DEHP on the germination and growth of *Triticum aestivum, Lepidium sativum and Brassica alba* according to OECD guideline 208. One nominal concentration (100 mg/kg dwt) was tested. DEHP was mixed with quartz sand and thereafter mixed with the test soil. For each species 20 seeds (4 parallels with 5 seeds) were exposed to DEHP contaminated soil and 20 seeds (4 parallels with 5 seeds) were used as control. No effects were seen on germination or growth during the 18 days the study lasted.

The bioavailability, and therefore the toxicity of a compound in soil, is dependent on soil characteristics, such as organic matter and clay content. Subsequently, to allow comparison of results from toxicity test carried out in different soils, test results has to be converted to a standard soil, which is defined as a soil with a organic matter content of 3.4%. The obtained NOECs could be normalised according to the formula:

$$\text{NOEC (or EC50)}_{standard} = \text{NOEC (or EC50)}_{experimen} * \frac{0.034}{\text{fract. org. matter in experimental soil}}$$

The organic carbon content of the soil in the study by Diefenbach (1998a) was reported to be < 1.5%. If 1.5% is assumed this corresponds to approx. 2.6 % organic matter. When applying the equation above on the NOEC (100 mg/kg) from this study a normalised NOEC of > 130 mg/kg dwt is obtained.

In the following studies plants (or parts of plants) were exposed to DEHP in water. Herring and Bering (1988) reported 40 and 50% reduction in seed germination for *Spinacia oleracea* and *Pisum sativum* respectively, when the seeds were soaked with water and DEHP at a nominal concentration of 0.1% (approximately 1,000 mg/l). Langebartels and Harms (1986) grew soybean and wheat cell cultures in nominal concentrations of DEHP up to 390 mg/l (added in methanol). No effect on the cell growth was observed. Effects of DEHP on passive membrane permeability in tissue disks of *Beta vulgaris* exposed to DEHP in water were studied in a short-term toxicity test (Schweiger et al., 1983). No effect was observed at 0.9 mg/l (the saturating concentration according to the authors). In these studies only nominal effect concentrations, far above the solubility level, were reported. These results can therefore not be used for the purpose to obtain a PNEC in this risk assessment. However, they indicate that DEHP is not harmful to plants.

3.2.3.2 Toxicity to soil invertebrates

The effect of DEHP on the earthworm, *Eisenia foetida*, was tested in a short-term contact test (Neuhauser et al., 1985) (see **Table 3.82**). No effect was observed at the highest concentration tested, 25 mg DEHP/cm^2 (nominal). DEHP was the least toxic compound among 44 tested. This result indicates that DEHP is not very harmful to soil organisms. The results cannot be used for a comparison with PECsoil, since the DEHP was added to a filter paper.

Diefenbach (1998b) studied the effects of DEHP on earthworm (*Eisenia foetida foetida)*. Fourty earthworms divided in four replicates were exposed to one nominal concentration (1,000 mg/kg dw) for 14 days. The earthworms were at least 2 months old weighing between 300 and 600 mg. DEHP was mixed with quartz sand and thereafter mixed with the test substrate whereafter water was added to achieve a water content of 35%. Temperature was kept at 20 ± 2 ^{o}C. No mortality or other effects were seen during the 14 days the study lasted. After the exposure period, the DEHP concentration in the earthworms was measured (see Section 3.1.1.4.3). From this study an unbounded NOEC of 1,000 mg/kg dwt is obtained. This NOEC cannot be normalised (see above) since the organic contents of the test soil was not reported.

Jensen et al. (2001) studied the effects of DEHP and DBP on the collembolan *Folsomia fimetaria*. Survival and reproduction on adult individuals (aged 23-26 days) were investigated by the use of small microcosms. The organisms were exposed for 21 days to DEHP in moist soil (< 1.5% organic carbon) at the nominal concentrations of 0, 1,000, 2,000, 3,000, 4,000 and 5,000 mg/kg dwt. The DEHP was added in acetone to dry soil. The acetone was evaporated and the soil remoistened with water before start of the experiment. Effects of the two phthalates on newly hatched collembolans were also tested in a multidish system. The endpoints were juvenile mortality, growth, and development (number of cuticles), the nominal test concentrations were 0, 100, 250, 500, and 1,000 mg DEHP/ kg soil (dwt), and the test duration was six weeks.

DEHP had no effect on any of the endpoints at the tested concentrations, maximum 5,000 and 1,000 mg/kg dwt for adults and juveniles respectively (DBP had marked effects at much lower concentrations). The study is considered reliable and the unbounded NOEC for the long-term test, starting with juveniles, is hence 1,000 mg/kg dwt. The organic carbon content of the soil in the study by was reported to be < 1.5%. If 1.5% is assumed this corresponds to approximately 2.6% organic matter. When applying the equation above on the NOEC (1,000 mg/kg) from this study a normalised NOEC of > 1,300 mg/kg dwt is obtained.

3.2.3.3 Toxicity to micro-organisms in soil

Three experimental studies involve possible effects on soil micro-organisms, exposed to DEHP in loam soil (see **Table 3.82**).

In an older study, Mathur (1974) investigated the short-term (8 hours) effects of DEHP on respiration in soils pre-exposed or not pre-exposed to 0.3% (by volume) DEHP. A nominal addition of 49 g DEHP /kg soil (0.2 ml DEHP to 4 g soil) resulted in inhibited respiration in the soil that had not been pre-exposed, while it enhanced the respiration in the pre-exposed soil. The procedure described for the respiration test (in Warburg flasks) leaves doubts regarding whether all the added DEHP was actually blended into the soil. Therefore, this study is considered invalid for the purpose of this risk assessment.

Cartwright et al. (1999) investigated the impact of phthalate plasticizers on soil microbial communities. They concluded that DEHP had no effect on the microbial community or membrane fluidity, even at 100 mg/g, and was predicted to have no impact on microbial communities in the environment. The only detected impact of DEHP on the microbial community was a (non-significant and non dose-related) reduction in numbers (84 % after incubation for 16 d) of *Pseudomonas sp.,* compared to the control. The DEHP was added to the test soils dissolved in methanol, and it is unclear if there was a solvent control. The added amount of methanol was very high, 0.05 ml per gram soil. Therefore this study is considered not valid for the purpose of this risk assessment.

In the third study (Kirchmann et al., 1991), the soil was incubated for 3 months with DEHP at 5 and 250 mg/kg. No effects, compared to control, were observed on soil biological processes (respiration, nitrogen mineralisation and, nitrification) at any of the test concentrations. A measured NOEC of 250 mg/kg is obtained. It does not say in the paper whether this value is reported per dry weight or per wet weight.

As a worst case, it is assumed that it is reported per dry weight. The organic carbon content in the experimental soil was 1.7% corresponding to approximately 2.9% organic matter. When applying the equation above (see Section 3.2.3.1) on the NOEC (250 mg/kg) from this study a normalised unbounded NOEC of about 300 mg/kg is obtained.

In a recent study (ECPI, 2000c**)** possible effects of DEHP on soil micro flora activity (measured as respiration), dehydrogenase activity, and nitrogen materialisation was investigated. Two soils, differing in e.g. organic contents (2.3 and 5.9% respectively) and particle size distribution, were tested. DEHP at concentrations 0, 10, 30, 100, 300 and 1,000 mg /kg dwt was tested in the respiration and dehydrogenase activity tests, while the DEHP concentrations in the nitrogen materialisation test were 0, 30, 300, 600 and 1,000 mg/kg dwt. The results were varying and therefore difficult to evaluate. There was marked non-dose related variation, both between the two soils and between the tests, in the recovery of added DEHP at start (42 - 117%). This fact adds question marks to these studies. At day 28 in soil with low organic contents, the recovery was 10-40% in the respiration and dehydrogenase tests while it was 49-74% in the nitrogen materialisation test. This indicates different degradation rates in the different tests. According to the authors, significant effects were only found for respiration and dehydrogenase activity at low organic contents and the highest dose (1,000 mg/kg dwt). Both parameters had increased at this test concentration. It is expected that these parameters correlate, since the enzyme dehydrogenase is activated in the respiration process. [However, the reported statistical test results for the dehydrogenase test (sometimes performed on a 1% significance level and sometimes on a 5% significance level) indicate that also other doses might differ significantly (on a 5% level) from the control.] An increase in these parameters may have three different

explanations: a) The added substance may act as a substrate for the microorganisms. This might be supported by high degradation rates, but in the present studies the degradation rate did not correlate with respiration or dehydrogenase activity. b) A part of the microorganism population may die from the treatment and other species in the population may take over and increase their biomass, partly by feeding on the dead organisms. c). The microorganisms are physically stressed by the added substance leading to increased respiration. Due to these different possibilities to interpret positive 'effects' in those tests, the results are very difficult to evaluate.

Nitrogen mineralisation is a sum parameter that includes several different processes among which nitrate production is known to be a sensitive process. No significant effects were observed in the nitrogen mineralisation test. However, statistical tests were only performed comparing data from day 28 with data from day 14. For soil with high organic contents, it appears that there might be differences when comparing nitrate production data at day 14 with day 0 data. In addition, a negative dose–response relation is indicated for nitrate production between day 28 and day 0 for this soil.

The authors have not discussed possible effects on different processes and different functional groups of the microorganism population. Such a discussion might have thrown some light on the variability in the results.

In conclusion, due to the variability in the recovery of DEHP and the variability and the question marks regarding the effects results, this study is not considered valid for determination of a $PNEC_{soil}$. However, a question mark still exists regarding possible effects on microorganisms (compare with sediment results Section 3.2.1.5.).

3.2.3.4 PNEC soil

Since no $PNEC_{water}$ was determined, the equilibrium partition method, given in TGD, could not be used to estimate $PNEC_{soil}$.

There are several studies in which plants or soil organisms have been exposed to DEHP. These studies indicate that DEHP is not harmful to soil organisms (and plants). However, most of these studies were questionable due to irrelevant exposure situations or nominal test concentrations above the solubility level. Only four studies exist with a relevant exposure situation that can be used for comparison with PECsoil. The four relevant studies represent three trophic levels. The lowest normalised unbounded NOEC from these studies was 130 mg/kg dwt (the highest tested concentration). If an assessment factor of 10 is applied on this NOEC, a PNEC of 13 mg/kg dwt is obtained. Since the PNEC is derived from a study where NOEC is the highest tested concentration, it can only be concluded that $PNEC_{soil} > 13$ mg/kg dwt.

Table 3.80 Acute toxicity to plants

Study No[4]	Test organism (Life stage)	T[1] St, Se, Fl[2] M, N[3]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure via water						**mg/l**	
1	*Beta vulgaris* red beetroot	St N?	?	24 hours	passive membrane permeability	NOEC LC_{50}	0.9 > 0.9	Nominal (?) concentrations: control and $2.3*10^{-3}$ mol/m^3, corresponding to 897 mg/m^3 The only concentration tested (the saturation concentration according to the authors). Effects on the permeability of the plasma membranes were investigated by changes in conductivity in the incubation medium. 24 other substances were also tested. The study was designed with the purpose to compare different test approaches with plants.

1) Temperature in ºC
2) Static, Semistatic or Flow through
3) Measured or Nominal concentrations
4) References: 1) Schweiger *et. al.*,1982

Table 3.81 Chronic (long term) toxicity to plants

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via air						**µg/cm^{-2}**	
1	*Sinapis alba* White mustard (5-8 leave stage)	Outdoor St M	tween 20	3-10 days	chlorosis	NOEC LOEC	8.75 > 8.75	Field study: The contaminants added by spraying plants in plots of $3*8m^{-2}$. Test concentration (measured in petri dishes placed in the sprayed area): untreated, Tween 20 at 1.05 and 3.5 µg/cm^{-2}, DEHP at 0.44, 2.19 and 8.75 µg/cm^{-2}.
2	*Brassica napus* (4-5 leave stage)	Outdoor St M	tween 20	3-10 days	chlorosis	NOEC LOEC	8.75 > 8.75	Field study: The contaminants added by spraying plants in plots of $3*8m^{-2}$.* Test concentration (measured in petri dishes placed in the sprayed area): untreated, Tween 20 at 1.05 and 3.5 µg/cm^{-2}, DEHP at 0.44, 2.19 and 8.75 µg/cm^{-2}.

Table 3.81 continued overleaf

Table 3.81 continued Chronic (long term) toxicity to plants

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. period	End-point	Effect conc.		Comment
	Exposure via soil					**mg/kg dw**		
3	*Spinacia oleracea*	23-25°C	-	14-16 days	seedling development	NOEC	?	Nominal conc.: control and 0.1% ??? 12 h light: 12 h dark It is unclear what the concentration really was. In the methods chapter it is said that 100 µg DEHP was added with 100 ml water to 100 g dried (170°C) soil (=> 1 mg/kg) but in the results the concentration discussed is 0.1 % which is said to have no effect on the plants. Therefore the test is considered invalid for the purpose of the risk assessment.
4	*Pisum sativum*	23-25°C	-	14-16 days	seedling development	NOEC	?	Nominal conc.: control and 0.1% ??? 12 h light: 12 h dark. It is unclear what the concentration really was. In the methods chapter it is said that 100 µg DEHP is added with 100 ml water to 100 g dried (170°C) soil (=> 1 mg/kg) but in the results the concentration discussed is 0.1% which is said to have no effect on the plants. Therefore the test is considered invalid for the purpose of the risk assessment
5	*Tricitum aestivum* *Lepidium sativum* *Brassica alba*	21.6 – 23.3°C	-	18 days	Germination and growth	NOEC LOEC	100 >100	Limit test, only one concentration tested. No effects were seen on either of the species tested.
	Exposure via water					**mg/l**		
6	*Spinacia oleracea*	N	methanol	13 days	seed germination	NOEC LOEC	< 1,000 1,000	Nominal concentrations: Control, methanol control, and 0.1% (corresponding to approximately 1,000 mg/l). A 50% reduction was observed compared to control. Methanol did not affect the germination.
7	*Pisum sativum*	N	methanol	13 days	seed germination	NOEC LOEC	< 1,000 1,000	Nominal concentrations: Control, methanol control, and 0.1% (corresponding to approx. 1000 mg/l). A 40% reduction was observed compared to control. Methanol did not effect the germination
8	*Glycine max* Soybean	28°C N	methanol	5 days	cell suspension growth	NOEC LOEC	390 > 390	Nominal concentrations: methanol control, 10^{-5}, $10^{-4.5}$, 10^{-4}, $10^{-3.5}$, 10^{-3} mol/L, corresponding to appr.0, 4, 12, 39, 123, 390 mg/l. Soybean cell suspension culture was used. The substance was added in 40-100 µL of methanol two days after start of the culture. The methanol control did not affect the growth.

Table 3.81 continued overleaf

Table 3.81 continued Chronic (long term) toxicity to plants

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure via water					**mg/l**		
9	*Triticum aestivum* Wheat	28°C N	methanol	5 days	cell suspension growth	NOEC LOEC	390 > 390	Nominal concentrations: control, 10^{-5}, $10^{-4.5}$, 10^{-4}, $10^{-3.5}$, 10^{-3} mol/L, corresponding to appr.0, 4, 12, 39, 123, 390 mg/l. Wheat cell suspension culture was used. The substance was added in 40-100 µL of methanol nine days after start of the culture. The methanol control did not affect the growth.

1) **T**emperature in ºC
2) **M**easured or **N**ominal concentrations
3) References:
1) Løkke and Rasmussen, 1983
2) Løkke and Rasmussen, 1983
3) Herring and Bering, 1988
4) Herring and Bering, 1988
5) Diefenbach, 1998a
6) Herring and Bering, 1988
7) Herring and Bering, 1988
8) Langerbartels and Harms, 1986
9) Langerbartels and Harms, 1986

Table 3.82 Toxicity to soil organisms

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure on filter paper					**mg/cm²**		
1	*Eisenia foetida*	20°C N	acetone or chloroform	48 h	survival	NOEC LOEC	25 > 25	At least five nominal concentrations up to 25 mg/cm², and a solvent control. Ten replicates.
	Exposure via soil					**mg/kg soil dw**		
2	*Eisenia foetida foetida*	20 ± 2 °C N	-	14 d	survival	NOEC LOEC	1,000 > 1,000	Limit test, only one concentration tested.

Table 3.82 continued overleaf

Table 3.82 continued Toxicity to soil organisms

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure via soil					**mg/kg soil dw**		
3	*Folsomia fimetaria* - adult - juvenile	20°C N	acetone	21 d	survival reproduction survival growth development	NOEC LOEC LOEC NOEC	5,000 > 5,000 1,000 > 1,000	Nominal concentrations: 1,000, 2,000, 3,000, 4,000, 5,000 mg/kg soil dw, and a solvent control. 10 females and 10males in each beaker, four replicates, test organisms fed bakers yeast twice, pH checked, 12 hours light: 12 hours dark. Nominal concentrations: 100, 250, 500, 1,000 mg/kg soil dw, and a solvent control. 20 juveniles in each multidish, 12 hours light: 12 hours dark.

1) **T**emperature in ºC
2) **M**easured or **N**ominal concentrations
3) References: 1) Neuhauser *et. al.,* 1985
2) Diefenbach, 1998b
3) Jensen *et.al.*, 2001

Table 3.83 Toxicity to terrestrial micro-organisms

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure via soil					**mg/kg**		
1	Natural soil flora	25°C M	-	3 m	Respiration, nitrogen mineralisation, nitrification	NOEC LOEC	250 > 250	Test concentrations: control, 5 and 250 mg/kg. Triplicates of each concentration. It is unclear whether the concentrations are reported per dry weight or per wet weight. The soil was dried sieved and stored (2 years) silt loam, (taken from the Swedish long-term fertility site at Fors). DEHP was uniformly mixed with a smaller portion of the dried soil. From this spiked soil known amounts were taken and mixed with more soil to reach the final concentration The soil was than wetted to optimal moisture content. Microorganisms were not added.
2	Natural soil flora	26°C N	-	8 hours	respiration inhibition	NOEC LOEC	< 49,150 49,150	Nominal DEHP concentration: 49,150 mg/kg (supposing a density for DEHP of 0.983 g/cm^3) and control. The DEHP (0.2 ml) and the soil (4 g) were added to different parts of a Warburg flask which was then carefully rolled around. The soil was Grenville loam from an experimental farm in Ottawa, with a moisture content equivalent to 66% field capacity. The soil was not pre-exposed to PAEs. Due to the high nominal concentration and the blending technique the study is considered invalid for the purpose of this risk assessment.

Table 3.83 continued overleaf

Table 3.83 continued Toxicity to terrestrial micro-organisms

Study No[3]	Test organism (Life stage, age)	T[1] M, N[2]	Vehicle	Exp. Period	End-point	Effect conc.		Comment
	Exposure via soil					**mg/kg**		
3	Natural soil flora	26°C N	-	8 hours	respiration inhibition	NOEC LOEC	49,150 >49,150	Nominal DEHP concentration: 49,150 mg/kg (supposing a density for DEHP of 0.983 g/cm^3) and control. The DEHP (0.2 ml) and the soil (4 g) were added to different parts of a Warburg flask which was then carefully rolled around. The soil was Grenville loam from an experimental farm in Ottawa, with a moisture content equivalent to 66% field capacity. The soil was pre-exposed to 0.3% DEHP (=2.95 g/kg) for 14 days in 22°C. The respiration rate was actually enhanced (30%) compared to control. Due to the high nominal concentration and the blending technique the study is considered invalid for the purpose of this risk assessment.
4	Natural soil flora	20°C (?) N	methanol	16 days	Number of bacteria, structural and functional diversity	NOEC LOEC	100,000 >100,000	DEHP was added, dissolved in methanol (0.05 ml/g soil), to soil concentrations at 0.1, 1, 10, and 100 g DEHP/kg dw. Exposure for 1, 7 and 16 days. The endpoints were total number of culturable bacteria and *Pseudomonas*, and structural and functional diversity of soil microbial community. Indications of 84% effects (non-significant) on numbers of *Pseudomonas*. The study is considered invalid for the purpose of this risk assessment due to the high addition of methanol and unclear description of treatment of controls.

1) **T**emperature in ºC
2) **M**easured or **N**ominal concentrations
3) References: 1) Kirchmann et. al. (1991)
   2) Mathur (1974)
   3) Mathur (1974)
   4) Cartwright et. al. (1999)

3.2.4 Secondary poisoning

3.2.4.1 Toxicity to birds

The toxicity data of DEHP to birds are presented in **Table 3.84**. Figures within brackets [x] below refer to the numbering of the studies in this table.

Toxicity tests exist for several species of birds where the exposure is via the feed. In four of the studies [1-4] the test substance actually is a mixture of DEHP and tetradecene or trichlorobenzene. It is not possible to distinguish between effects caused by DEHP and effects caused by the other substance, or combination effects. Therefore, the results from these studies are not directly used for deriving a NOEC in this risk assessment.

Effects on lipid metabolism are shown in two studies with lowered plasma lipid concentrations and lowered cholesterol, among other effects, at relatively high concentrations of DEHP (10,000 – 20,000 ppm) [7, 8]. In another study, wild starlings gained more weight and showed an increase in lipid deposition at low concentrations of DEHP (25 ppm). However, it seems difficult to interpret the findings in the latter study as adverse effects with ecological relevance. Hence, this study is not used for deriving a PNEC in this risk assessment.

In three studies on hens at relatively high concentrations of DEHP adverse effects on egg production was shown [7-9]. In a long term study on White Leghorn Hen exposed to DEHP for about 230 days, egg production ceased and abnormality of ovaries and hypertrophy of liver and kidneys were found at the lowest test concentration, LOEC = 5,000 ppm (Ishida et al., 1982). This is the lowest effect value regarding toxicity to birds (when the starling study mentioned above is discounted). According to TGD a NOEC can be calculated as LOEC/2, when effect percentage is 10-20. The prerequisite of 10-20% is not fulfilled in this case, since the effect was 100%. In another study on hens exposed to DEHP for 28 days decreased egg production (14%) and effects on lipid metabolism were found at the lowest test concentration, LOEC = 10,000 ppm (Wood and Bitman 1980). An effect level of 14% fulfil the prerequisite for dividing the LOEC by two, resulting in a NOEC of 5,000 ppm. The feed in this study was a standard laying mash (consisting mainly of different meals). In TGD (Appendix VII) it is stated that the energy content of grain is higher than fish. This means that in order to obtain the same amount of energy more wet weight of fish must be consumed compared to grain. Therefore a correction factor of 3 may be applied for the difference in caloric content of the diet of laboratory animals and the diet of fish-eating birds or mammals". Hence, the NOEC can be lowered with a factor 3 resulting in a NOEC of about 1,700 mg/kg food.

3.2.4.2 $PNEC_{oral}$ for secondary poisoning

Effects on birds and mammal populations are rarely caused by mortality after short-term exposure. Therefore, results from long-term studies are preferred, such as NOECs for mortality, reproduction or growth. According to TGD, an assessment factor of 100 can be applied to the NOEC for a 28-day repeated dose test to derive a PNEC. When chronic studies are available an assessment factor of 10 may be used. Reproduction toxic effects are regarded as chronic effects and for these the same assessment factor (10) may be used.

For birds there is one study with results suitable for deriving a PNEC. This is the 28-day study with a NOEC of 1,700 mg/kg food for reproductive effects calculated from a LOEC of

10,000 ppm. Since it is a reproductive effect strictly applying TGD would lead to the use of an assessment factor of 10. However, there is also a long-term study (230 days exposure) in which egg laying was totally impaired at a dose of 5,000 ppm. From this study no NOEC can be derived but the results imply that a larger assessment factor than 10 is needed for the derivation of PNEC from the 28-day study. Therefore, an assessment factor of 100 is chosen resulting in a PNEC of 17 ppm. This PNEC will be used in the risk characterisation for secondary poisoning of birds feeding on mussels.

There are several studies on mammalians exposed to DEHP via oral exposure (see Section 4.2.9). In a continuous breeding study (chronic, > 90 days) on mice, Lamb et al. (1987) found that 1,000 ppm DEHP produced a dose dependent and significant decrease in the number of litters as well as the number and proportion of pups born alive. The NOAEL was 100 ppm DEHP in food. Impairment of fertility is considered to be an ecologically relevant effect. Applying an assessment factor of 10 to the NOAEL from this study results in a PNEC of 10 ppm.

In another chronic study, minimal to mild Sertoli cell vacuolisation was observed in testes of young male rats, which had been given 500 ppm DEHP in the diet for 13 weeks (Poon et al., 1997). At the next higher concentration, 5,000 ppm, the testis weights were significantly reduced, and microscopic examination revealed a mild to moderate, bilateral, multifocal or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis. Hence, preliminary stage of reproductive impairment was shown already at a dose of 500 ppm. Applying an assessment factor of 10 to the NOAEL (50 ppm) for this effect results in a PNEC of 5 ppm.

Irreversible testicular damage was shown in male rats exposed *in utero* and during suckling at very low dose levels. The NOAEL was 4.8 mg/kg bw/day at a food concentration of 100 ppm (Wolfe et al., 2003). According to TGD a food conversion factor of 3 should be applied, resulting in a $NOEC_{oral,\ mammalianss}$ of 100 ppm/3= 33.3 ppm. Finally, when applying the assessment factor of 10 (for chronic studies according to TGD) a PNEC of 3.3 ppm is obtained.

In conclusion, for the purpose of this risk assessment the $PNEC_{oral,\ mammalians}$ for non-compartment specific effects relevant to the food chain is set to 3.3 ppm.

Table 3.84 Toxicity to birds

Study No[1]	Test organism (Life stage, age)	Vehicle	Exp. period	End-point	Effect conc. (mg/kg)		Comment
1	*Anas Platyrhynchos* (ducklings, 10 days)	corn oil	5 days	mortality	LC_{50} NOEC	> 100,000 100,000	The test substance was applied in the diet at concentrations of 3,125, 6,250, 12,500, 25,000, 50,000 and 100,000 ppm. The treated groups were compared with three control groups. Eight animals were used per group. None of the animals died in any of the groups. The body weight gains were dose-dependently reduced in all treatment groups except the lowest dose group. Food consumption was dose-dependently reduced in the three highest dose groups but normalised during a 3-days post treatment period, indicating that the ducklings avoided the food. The test substance which was identified by the code name LL-1131 was a mixture of two substances (according to IUCLID, only the code name is given in the available report): di-(2-ethylhexyl) phthalate at 90% and tetradecene at 10%
2	*Anas Platyrhynchos* (ducklings, 10 days)	corn oil	5 days	mortality	LC50 NOEC	> 40,000 40,000	The test substance was applied in the diet at concentrations of 2,013, 3,660, 6,655, 12,100, 22,000 and 40,000 ppm. The treated groups were compared with three control groups. Eight animals were used per group. None of the animals died in any of the groups. The body weight gains were dose-dependently reduced in all treatment groups (9% at the lowest concentration). Food consumption was dose-dependently reduced in the four highest dose groups but normalised during a 3-days post treatment period(except the two highest dose groups). The test substance which was identified by the code name LL-1132 was a mixture of two substances (according to IUCLID, only the code name is given in the available report):-(2-ethylhexyl) phthalate at 70% and trichlorobenzene at 30%
3	*Phasianus colchicus* "ring-necked" pheasants (10 d)	corn oil	5 days	mortality	LC50 NOEC	40,000 < 2,013	The test substance was applied in the diet at concentrations of 2,013, 3,660, 6,655, 12,100, 22,000 and 40,000 ppm. The treated groups were compared with three negative control groups and positive control groups, which were fed dieldrin treated food at different concentrations. Eight animals were used per group None of the animals died in the 6,655 and 22,000 ppm groups, 1/8 animal died in the 2,013, 3,660 and 12,100 ppm group and 4/8 animals died in the 40,000 ppm group. Mortality occurred between day 2 and 4 of the treatment in the highest dose group and at day 5 in the other groups. Body weights were reduced showing a trend to dose-response. Food consumption was reduced in all treatment groups with during a 3-days post treatment period (except the highest dose group). The test substance which was identified by the code name LL-1131 was a mixture of two substances (according to IUCLID, only the code name is given in the available report): di-(2-ethylhexyl) phthalate at 90% and tetradecene at 10%.

Table 3.84 continued overleaf

Table 3.84 continued Toxicity to birds

Study No[1]	Test organism (Life stage, age)	Vehicle	Exp. Period	End-point	Effect conc. (mg/kg)		Comment
4	*Phasianus colchicus* "ring-necked" pheasants (10 d)	corn oil	5 days	mortality	LC50 NOEC	9,000 4,222	The test substance was applied in the diet at concentrations of 2,406, 4,222, 7,407, 12,996, 22,800 and 40,000 ppm. The treated groups were compared with three negative control groups and positive control groups, which were fed dieldrin treated food at different concentrations. Eight animals were used per group. None of the animals died in the 2,406 and 4,222 ppm groups, 3/8 animals died in the 7,407 ppm group and 8/8 animals die in the 12,996, 22,800 and 40,000 ppm groups. Mortality occurred between day 2 and 5 of the treatment. Body weight gains were dose-dependently reduced. Food consumption was reduced in all treatment groups with normalisation during a 3-days post treatment period. The test substance which was identified by the code name LL-1132 was a mixture of two substances (according to IUCLID, only the code name is given in the available report):-(2-ethylhexyl) phthalate at 70% and trichlorobenzene at 30%
5	*Streptopelia risoria* ring dove (eggs)	?	?	eggshell thickness, breaking strength, permeability, shell structure	LC50 NOEC	> 10 > 10	DEHP was applied in the diet at a concentration of 10 ppm with a control group. Pairs of Ringdoves were maintained in individual cages and their eggs were examined. Di-n-butyl phthalate was also tested in this study at a concentration of 10 ppm in the diet. Eggshell thickness was decreased and permeability to water increased by the diet containing DINBP, whereas no significant effect was found with DEHP.
6	*Sturnus vulgaris* Starling (adults)	corn oil	30 days	weight increase, lipid content	NOEC LOEC	< 25 25	DEHP was applied in the diet at concentrations of 25 and 250 ppm with a control group. Twenty-one wild captured adult starlings of mixed sexes were used per group. At 30 days of exposure eight birds were removed and examined. The remaining birds were provided untreated food for a 14 days cleanup period. The DEHP-dosed starlings gained more weight than birds in the control group and showed an increase in lipid deposition. The increases in body weights and carcass lipids were not accompanied by increases in food consumption. No other treatment-related were detected at 30 days exposure.

Table 3.84 continued overleaf

Table 3.84 continued Toxicity to birds

Study No[1]	Test organism (Life stage, age)	Vehicle	Exp. period	End-point	Effect conc. (mg/kg)		Comment
7	*Gallus domesticus* Hen (young egglaying)	-	28 days	feed consumption, egg production, plasma lipids, liver lipids	NOEC LOEC	< 10,000 10,000	DEHP was applied in the diet at a concentration of 1% with a control group. A group fed DEHP and tallow at concentrations of 1% and 5% respectively was also tested in this study. Tallow was added to insure that enough lipids were available for egg production in the event DEHP exerted severe lipid lowering effects. Fifteen hens were used per group. Both diets containing DEHP lowered feed consumption 10% but did not significantly affect body weight. Both diets decreased egg production for the 4-wk experimental period (14% and 5% with and without tallow respectively). However, egg weight, egg composition (percent shell, white, and yolk), yolk lipid and cholesterol of eggs showed no differences compared to control eggs. Total liver lipid increased in both diets (54% and 19% with and without tallow respectively). Total cholesterol in livers increased (81% and 29% with and without tallow respectively). The fat content of pectoralis major muscle decreased significantly. Both diets lowered plasma lipid concentration about 20% and free and total cholesterol 20-30%.
8	*Gallus domesticus* Hen (young egglaying)	-	28 days	body weight, feed consumption, egg production, plasma lipids, liver lipids	NOEC LOEC	< 20,000 20,000	DEHP and three structurally related side-chain analogs, 2-ethylhexanol (2-EH), 2-ethylhexanal (2-EHALD), 2-ethylhexanoic acid (EHA), were fed to 5 treatment groups of 8 laying hens and one control group at concentrations of: DEHP 2%, 2-EH 1%, 2-EH 2%, 2-EHALD 1%, 2-EHA 1%. Experimental treatments lowered feed consumption, body weight, and egg production. The DEHP and side-chain analog diets lowered plasma total lipids. DEHP 2%, 2-EH 2%, and 2-EHA 1% diets lowered plasma free cholesterol. Liver weights were not significantly changed, but liver fat was reduced. Hen egg yolk percent fat, lipid classes, and free and total cholesterol were unaffected by any experimental treatment.
9	*Gallus domesticus* White Leghorn Hen (10-month-old)		230 days	egg production, abnormal ovaries, liver or kidneys	NOEC LOEC	< 5,000 5,000	DEHP was applied in the diet at concentrations 0 (control), 0.5 and 1g DEHP/100 g feed. Egg production was stopped and abnormality of ovaries and hypertrophy of liver and kidneys were found in the DEHP administration groups. It was recognised that DEHP has a tendency to accumulate selectively in feathers and adipose tissue.
10	*Gallus domesticus* White Leghorn Hen (10-month-old)		25 days	egg production, abnormal ovaries, liver or kidneys	NOEC LOEC	> 2,000 > 2,000	DEHP was applied in the diet at concentrations 0 (control) and 0.2g DEHP/100 g feed. All of the hens continued to lay eggs, and the livers, kidneys, and ovaries did not change.

Table 3.84 continued overleaf

Table 3.84 continued Toxicity to birds

Study No[1]	Test organism (Life stage, age)	Vehicle	Exp. period	End-point	Effect conc. (mg/kg)		Comment
11	*Phasianus colchicus* Ring-necked pheasant (10 days old)	corn oil	5 days (8 days)	mortality	LC50 NOEC	> 5,000 > 5,000	Three concentrations of which the highest was 5,000 mg/kg. The DEHP was added to the feed with corn oil. A corn oil control was included. 131 chemicals were tested. The DEHP was only tested in a range-finding test, since no mortality was observed at the highest tested concentration. 8 days test: 5 days of exposure to treated diet followed by 3 days of untreated diet
12	*Anas platyrhyncos* Mallard (10 days old)	corn oil	5 days (8 days)	mortality	LC50 NOEC	> 5,000 > 5,000	Three concentrations of which the highest was 5,000 mg/kg. The DEHP was added to the feed with corn oil. A corn oil control was included. 131 chemicals were tested. The DEHP was only tested in a range-finding test, since no mortality was observed at the highest tested concentration. 8 days test: 5 days of exposure to treated diet followed by 3 days of untreated diet

1) References:

1) General Electric Co. (1976a)
2) General Electric Co. (1976)
3) General Electric Co. (1976b)
4) General Electric Co. (1976b)
5) Peakall (1974)
6) O´Shea and Stafford 1980
7) Wood and Bitman (1980)
8) Wood and Bitman (1984)
9) Ishida et al. (1982)
10) Ishida et al. (1982)
11) Hill et.al. (1975)
12) Hill et.al. (1975)

3.2.5 Other effects

3.2.5.1 Endocrine disruption

Possible effects of DEHP on the normal functioning of the endocrine system have been investigated in a number of studies summarised in Section 4.1.2.9.3. Both in vivo and in vitro study results indicate that DEHP can interfere with the endocrine function and also influence the sexual differentiation (e.g. Gray et al., 1999 and Jones et al 1993). Due to the effects on the Leydig cells as measured by a decreased testosterone output, it cannot be excluded that DEHP may exert an antiandrogen effect. The results of recently performed in vivo studies in rats exposed to DEHP or DBP support the hypothesis that exposure to phthalates may be provoked by an antiandrogen mechanism (Gray et al., 1999, Mylchrest and Foster, 1998).

Norrgren et al. (1999), studied the effects on sexual differentiation in Atlantic salmon (S*almo salar*) exposed to DEHP, nonylphenolethoxilate (NPEO) or 17ß-estradiol via the food (see Section 3.2.1.1). The control group consisted of 49% females while the fish fed diets containing15 and 30 mg/kg 17ß-estradiol developed into 88 and 100% phenotypic females, respectively. The number of females in the groups fed DEHP contaminated food was similar to the control, 47% females, in the low dose, (300 mg DEHP/ kg food dwt) while in the high dose (1,500 mg/kg food dwt) it was significantly higher 64%, ($P < 0.05$ chi-square test).

In addition to the feeding trial juvenile salmon weighing approximately 7.5 g were injected intraperitoneally with different test compounds in order to study induction of vitellogenin synthesis. Each group consisted of 5 fish that were kept in separate aquaria (50 l). The group injected with DEHP received a total dose of 160 mg kg^{-1} during 17 days. At termination of the exposure period blood was sampled from the fish and analysed for vitellogenin. No vitellogenin was detected in the blood of the DEHP injected fish.

3.2.5.2 MEHP

Mono (2-ethylhexyl) phthalate (MEHP) is the major metabolite when DEHP is degraded in the environment as well as in biota although the rate of conversion/metabolism differ between different environmental compartments as well as different species (see Section 3.1.2.4, 3.1.5.1, and 4.1.2.1). MEHP has proven to be toxic in studies with mammals and the substance could be responsible for many of the effects seen in toxicity studies with DEHP (see Section 4.1.2.6 - 4.1.2.9). It is therefore reasonable to believe that MEHP will be toxic also to other species like birds, fish, frogs etc. However no such information is available. A literature search did not reveal any toxicity studies on other species than those reviewed in Section 4 - Human Health.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

3.3.1.1 Micro-organisms in sewage treatment plants

Only one study was considered valid to be used for the risk assessment of STP. In this study no effect on respiration was observed at the highest tested concentration (2007 mg/l). Therefore the only conclusion that can be drawn is that $PNEC_{STP}$ is > 201 mg/L (see Section 3.2.1.8).

The PECs for STP are presented in **Table 3.85**. Reported measured site-specific data for processing sites did not include any information on the inflow to the STP, therefore no site-specific local risk characterisation for STP has been done for the processing/conversion step. However, due to the small emissions to waste water from these sites it can be concluded that the PEC/PNEC ratios would be far below 1.

Table 3.85 PEC/PNEC ratios, STP

life c. stage	Description		PEC mg/l		PEC PNEC[1]
1a	Production site 1		0.04	<	0.0002
1a	Production site 2		0.03	<	0.0002
1a	Production site 3		0.005	<	0.00003
1a	Production site 4		0.0009	<	0.000004
1a	Production site 5		0.00008	<	0.0000004
1a	Production site 6		20	<	0.1
1a	Production site 7		0.0002	<	0.0000008
1a	Production site 8		0.1	<	0.0005
1a	Production site 9		0.01	<	0.00006
1a	Production site 10		0		0
1a	Production site 11		0.2	<	0.001
1a	Production site 12		2.7	<	0.01
2a	Polymer-Calendering		0.1	<	0.0006
2b	Polymer-Extrusion comp		0.04	<	0.0002
2c	Polymer-Extrusion prod		0.04	<	0.0002
2d	Polymer -	with air cleaning	0.05	<	0.0003
	Plastisol spread coating	without air cleaning	0.43	<	0.002

Table 3.85 continued overleaf

Table 3.85 continued PEC/PNEC ratios, STP

life c. stage	Description		PEC mg/l		PEC PNEC[1]
2e	Polymer	with air cleaning	0.03	<	0.0001
	Other plastisol	without air cleaning	0.23	<	0.001
3a	Sealants/adhesives etc.	formulation	1.3	<	0.006
3b		processing	0	<	0
4a	Lacquers and paint	formulation	0.16	<	0.0008
4b		processing	0.01	<	0.00006
5a	Printing ink	formulation	0.19	<	0.0009
6a	Ceramics	formulation	0.007	<	0.00003
7	Municipal STP		0.03	<	0.0001
8a	Paper recycling		0.04	<	0.0002
8b	Disposal - Car shredding		0		0
8c	Disposal - waste incin.		0		0

1) PNEC > 200 mg/l.

The PEC levels are in many cases higher than the "true" water solubility (3 ug/L). Even the "apparent solubility" is exceeded in some cases. Two production sites (6 and 12) show the highest concentrations. When DEHP arrives in the treatment plant most of it (approximately 90%) adsorbs to the sludge. The same situation is probably the case in toxicity tests with sludge. Since the bacteria are supposed to grow on the organic material in the effluents, they are also exposed to chemicals associated with this material.

The information on how the specific formulation sites are connected to treatment plants is sparse. They may be connected to municipal STPs and the processing/formulation may occur intermittently.

For all of the scenarios the PEC/PNEC ratios are below 1. **Conclusion (ii)**.

3.3.1.2 Surface waters

Short term and/or long term effect studies on aquatic organisms, exposed to DEHP via water, are available for fish, amphibians, aquatic invertebrates, algae, higher plants, and micro-organisms. However, there are no reliable long-term studies below the "apparent water solubility" of DEHP indicating effects on organisms exposed to DEHP in water only. Hence, a $PNEC_{water}$ cannot be specified. However, effects are shown on fish exposed to DEHP via food only. Therefore a $PNEC_{food}$ for fish has been determined: 16 mg/kg (fresh food) (see Section 3.3.4, "Secondary poisoning").

The Local PECs based on the estimated (EUSES) PECregional (2.2 µg/l) and PECregional(0.8 µg/l) derived from measured data, respectively are presented in **Table 3.86**.

Table 3.86 Local PECs based on EUSES-estimated PECregional (2.2 µg/l) and PECregional (0.8 µg/l) derived from measured values

Life cycle stage	Local PEC based on estimated PECregional (µg/l)	Local PEC based on measured PECregional (µg/l)
1a Production		
site 1	2.2	0.85
site 2	3.3	1.9
site 3	2.2	0.83
site 4	2.3	0.87
site 5	2.2	0.81
site 6	22	20
site 7	2.2	0.80
site 8	2.6	1.2
site 9	2.2	0.81
site 10	2.2	0.80
site 11	18	17
site 12	220	219
2 Polymer form/proc.		
2a-Calendering – generic	11	9.8
site F3	2.3	0.8
site S4	2.2	0.8
site S6	2.4	0.9
2b Extrusion comp – generic	5.6	4.2
site S5	2.2	0.8
2c Extrusion prod – generic	5.6	4.2
site F2	2.5	1.0
site F7	2.3	0.9
site I9	2.4	1.0
site S11	2.2	0.8
2d Plastisol spread coating		
with air cleaning-generic	6.3	4.9
without air cleaning-generic	37	36
site F1	2.2	0.8
site S8	2.2	0.8
2e Other plastisol		
with air cleaning	4.4	3.0
without air cleaning	21	19
Not known site ES12	2.2	0.8
site ES13	2.2	0.80

Table 3.86 continued overleaf

Table 3.86 continued Local PECs based on EUSES-estimated PECregional (2.2 µg/l) and PECregional (0.8 µg/l) derived from measured values

Life cycle stage		Local PEC based on estimated PECregional (µg/l)	Local PEC based on measured PECregional (µg/l)
3-6 Non polymer form/proc.			
3a Sealants/adhesives	formulation – generic	104	102
	Adhesives site F10	2.2	0.8
3b Sealants/adhesives	processing – generic	2.2	0.8
4a Laquers and paints	formulation – generic	15	14
4b Laquers and paints	processing – generic	3.2	1.6
5a Printing inks	formulation – generic	17	16
6 Ceramics	formulation – generic	2.8	1.2
7 Municipal STP – generic		4.8	3.2
8a Paper recycling – generic		5.2	3.6
8b Car shredding – generic		2.2	0.8
8c waste incineration. – generic		2.2	0.8

Due to the absence of effects at or below the water solubility of DEHP, there seems to be no concern for aquatic organisms exposed to DEHP via the water phase only, and there is no need for further testing with exposure via the water phase alone. However, studies with exposure via the food indicate effects on fish (see Section 3.3.4, "Secondary posisoning"). **Conclusion (ii)**.

3.3.1.3 Sediment dwelling organisms

Several studies exist in which organisms are exposed to DEHP in sediment. These studies involve organisms from several trophic levels: micro-organisms, detritivorous and predatory insect larvae, and amphibians and indicate that DEHP is not harmful to sediment organisms. An overall PNECsediment of > 100 mg/kg dwt, based on an embryo /larvae study with amphibians (frogs) where no effects were seen at the highest test concentration (1,000 mg/kg dwt), is chosen to be used in the risk assessment.

The PEC/PNEC ratios for sediment dwelling organisms are presented in **Table 3.87**. For comparison PECsediment based both on calculated and measured PECregional surface water are shown. For the local PECs based on reported site-specific data, only the PECs based on measured PECregional are shown.

Table 3.87 PEC/PNEC ratios for the sediment compartment

Life cycle stage		Local PEC based on estimated $PEC_{regional}$[2] (mg/kg dwt)	PEC PNEC[1]	Local PEC based on measured $PEC_{regional}$[3] (mg/kg dwt)	PEC PNEC[1]
1a Production					
	site 1	21	< 0.2	7.9	< 0.08
	site 2	31	< 0,3	17	< 0.2
	site 3	21	< 0.2	7.7	< 0.08
	site 4	21	< 0.2	8.1	< 0.08
	site 5	21	< 0.2	7.5	< 0.08
	site 6	204	< 2.0	191	< 2
	site 7	21	< 0.2	7.5	< 0.08
	site 8	24	< 0.2	11	< 0.1
	site 9	21	< 0.2	7.6	< 0.08
	site 10	21	< 0.2	7.5	< 0,07
	site 11	168	< 1.7	155	< 1,6
	site 12	2058	< 21	2045	< 20
2 Polymer form/proc.					
2a-Calendering – generic		104	< 1.0	91	< 0.9
	site F3	-	-	7.8	< 0.08
	site S4	-	-	7.7	< 0.08
	site S6	-	-	9.0	< 0.09
2b Extrusion comp – generic		52	< 0.5	39	< 0.4
	site S5	-	-	7.5	< 0.07
2c Extrusion prod – generic		52	< 0.5	39	< 0.4
	site F2	-	-	10	< 0.1
	site F7	-	-	8.2	< 0.08
	site I9	-	-	9.2	< 0.09
	site S11	-	-	7.5	< 0.07
2d Plastisol spread coating	*with air cleaning - generic*	59	< 0.6	46	< 0.5
	without air cleaning - generic	345	< 3.5	332	< 3.3
	site F1	-	-	7.7	< 0.07
	site S8	-	-	7.8	< 0.08
2e Other plastisol	*with air cleaning – generic*	41	< 0.4	28	< 0.3
	without air cleaning – generic	194	< 1.9	181	< 1.8
Not known	site ES12	-	-	7.5	< 0.07
	site ES13	-	-	7.7	< 0.08

Table 3.87 continued overleaf

Table 3.87 continued PEC/PNEC ratios for the sediment compartment

Life cycle stage		Local PEC based on estimated $PEC_{regional}$[2] (mg/kg dwt)	PEC PNEC[1]	Local PEC based on measured $PEC_{regional}$[3] (mg/kg dwt)	PEC PNEC[1]
3-6 Non polymer form/proc.					
3a Sealants/adhesives	formulation – generic	966	< 9.7	951	< 9.5
	Adhesives site F10	-	-	7.5	< 0.07
3b Sealants/adhesives	processing – generic	21	< 0.2	6.0	< 0.06
4a Lacquers and paints	formulation – generic	144	< 1.4	129	< 1.3
4b Lacquers and paints	processing – generic	30	< 0.3	15	< 0.15
5a Printing inks	formulation – generic	162	< 1.6	149	< 1.5
6 Ceramics	formulation – generic	26	< 0.3	11	< 0.1
7 Municipal STP – generic		44	< 0.4	30	< 0.3
8a Paper recycling – generic		48	< 0.5	33	< 0.3
8b Car shredding – generic		21	< 0.2	6.0	< 0.06
8c waste incineration. – generic		21	< 0.2	6.0	< 0.06

1) PNEC > 100 mg/kg dw
2) Local PECsediment based on PECregional surface water estimated by EUSES (2.2µg/l)
3) Local PECsediment based on PECregional surface water derived from measured data (0.8µg/l)

Conclusion

DEHP is slowly degraded in the sediment due to low oxygen levels. This in combination with high ability to adsorb to organic matter contributes to high concentrations in sediments.

The PEC/PNEC ratios are in most cases below 1. For some scenarios - production sites 6, 11 and 12 the ratios are higher than 1. These sites have however, ceased production. For the generic local scenarios 2d plastisol spread coating without air cleaning, 2e other plastisol without air cleaning, 3a sealants formulation, 4a lacquers and paint formulation, and 5a printing inks formulation - the ratios are also higher than 1. The PECs for these scenarios are calculated based on default emission factors from the emission scenario document (ESD) on Plastics additives. For the scenarios based on site-specific data in the formulation and processing stages, the PEC/PNEC ratio is 0.1 or less.

The ESD on Plastics additives suggests that spread-coating sites without air-cleaning release ten times more than an otherwise equivalent site with air-cleaning. Half of the release is supposed to go to waste water. The site specific information from the two spread coating sites (F1 and S8) indicates that the emissions to waste water from spread coating are much lower than what the ESD suggests.

For sealants/adhesives formulation the situation is similar. The site specific information (site F10) indicates much lower emissions to waste water than suggested by the generic release estimation based on default emission factors. The site specific risk characterisation gives a PEC/PNEC ratio below 0.1, which is two orders of magnitude lower than for the generic local scenario (3a sealants formulation).

However, site-specific data is limited to a few out of many sites (in the order of 20 out of 800). The volume used by these sites covers only a few percent of the total volume used in these use areas. The data can therefore not be taken as representative for all other sites (see also

Section 3.1.1.2.1). As a realistic worst case it has been assumed that there are plastisol spread coating and sites formulating lacquers, paints, printing inks, sealant and/or adhesives that have emissions of a magnitude as indicated by the rather recently agreed ESD, thus leading to PEC/PNEC ratios > 1.

The PNEC used (> 100 mg/kg) is derived from a study on frog eggs where no effects were seen at the highest tested concentration. With the exception of a study on microorganisms, which was not considered relevant to use for deriving a PNEC, all other studies on sediment organisms indicate even higher no effect levels. For *Chironomus spp*, no effects were seen up to 11,000 mg/kg dwt. The actual PNEC may thus be higher. Repeating the tests at higher test substance concentrations than those used may refine the PNEC. This option is not chosen because data is already available for DEHP and because of the difficulties associated with testing very high concentrations of a substance. Alternatively more data on emissions and concentrations in sediment could be collected. Since a refinement of the exposure assessment may result in a **conclusion (ii)** for this compartment further information could be requested. However since the same scenarios that have PEC/PNEC ratios > 1 for sediment-dwelling organisms, also have PEC/PNEC ratios > 1 for the food chains based on aquatic organisms, the risk reduction strategy will address the emissions in these scenarios anyway. Further studies are therefore not requested at this point.

Therefore, for these uses a **conclusion (i)** "on hold" is drawn.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for the other environmental spheres will eliminate the need for further information on sediment dwelling organisms.

3.3.2 Atmosphere

No studies exist from which a $PNEC_{atmosphere}$ (to compare with $PEC_{atmosphere}$) could be derived.

However, NOEC values were estimated for single applications of DEHP on foliage of plants (87.5 mg/m^2) and on the venter of abdomen of housefly (about 200 mg/m^2, supposing that the housefly has an area of 1 cm^2, compare Section 3.2.2.2). These NOECs could be compared with the measured and estimated daily deposition rates accounted for in Sections 3.1.6.3 and 3.1.6.3.1 and Annex 1. The highest measured deposition rate is 9.4 µg/m^2 · day, and the highest calculated (EUSES) deposition rate (life cycle stage 3a sealants, adhesives formulation) is 71 µg/m^2 · day. This means that to reach the NOEC value (single occasion deposition) for plants, 87.5 mg/m^2, about 1,000 days at the highest calculated deposition rate, and about 9,000 days at the highest measured deposition rate, is needed. This is to reach a NOEC that is based on the highest tested concentration. From this calculation we conclude that it is not likely that atmospheric exposure/deposition of DEHP, caused by activities dealt with in this risk assessment, would have direct effects on organisms exposed to DEHP directly from the atmosphere. **Conclusion (ii)**

There are no data indicating risk for the atmosphere compartment.

3.3.3 Terrestrial compartment

Several studies exist in which plants or soil organisms have been exposed to DEHP. These studies indicate that DEHP is not harmful to soil organisms (incl. plants). The NOECs from tests that were considered valid for the risk assessment were all the highest tested concentrations.

Since these studies each represent one trophic level, the lowest NOEC (from a plant test) was chosen to estimate PNEC. Hence, a $PNEC_{soil}$ > 13 mg/kg dwt is derived (see Section 3.2.3.4).

Agricultural soil

The PEC/PNEC ratios are presented in **Table 3.88**.

Table 3.88 Comparison of calculated PEC_{local} for agricultural soil with $PNEC_{soil}$

Life cycle stage		**PEC** (mg/kg dwt)	**PEC / PNEC[1]**
2 Polymer form/proc.			
2a Polymer-Calendering –generic		9.2	< 0.7
	site F3	0.06	< 0.005
	site S4	0.06	< 0.005
	site S6	0.2	< 0.02
2b Polymer-Extrusion comp – generic		3.5	< 0.3
	site S5	0.02	< 0.005
2c Polymer-Extrusion prod – generic		3.5	< 0.3
	site F2	0.3	< 0.02
	site F7	0.1	< 0.01
	site I9	0.2	< 0.02
	site S11	0.02	< 0.005
2d Polymer – Plastisol spread coating	*with air cleaning – generic*	4.2	< 0.3
	without air cleaning – generic	354	< 2.7
	site F1	0.05	< 0.005
	site S8	0.09	< 0.007
2e Polymer - Other plastisol	*with air cleaning – generic*	2	<0.2
	without air cleaning – generic	19	<1.5
2? Not known	site ES12	0.02	<0.005
	site ES13	0.04	<0.005
3-6 Non polymer form/proc.			
3a Sealants/adhesives	formulation – generic	103	< 7.9
	Adhesives site F10	0.03	< 0.005
3b Sealants/adhesives	processing – generic	0.02	< 0.001
4a Lacquers and paints	formulation – generic	13	< 1.0
4b Lacquers and paints	processing – generic	1	< 0.1

Table 3.88 continued overleaf

Table 3.88 continued Comparison of calculated PEC_{local} for agricultural soil with $PNEC_{soil}$

Life cycle stage		PEC (mg/kg dwt)	PEC / PNEC[1]
5a Printing inks	formulation – generic	15.5	< 1.2
6a Ceramics	formulation – generic	0.5	< 0.04
7 Municipal STP – generic		2.6	< 0.2
8a Paper recycling – generic		3	< 0.2
8 Disposal - Car shredding – generic		0.02	< 0.001
8c Disposal - waste incin. – generic		0.02	< 0.001
Regional		0.07	< 0.005

1) PNEC > 13 mg/kg dwt

Conclusion

The PEC/PNEC ratios are higher than 1 for agricultural soil in three of the scenarios, 2d plastisol spread coating without air cleaning, 2e other plastisol without air cleaning, and 3a sealants formulation. The PECs for these scenarios are calculated based on default emission factors from the emission scenario document (ESD) on Plastics additives. For the scenarios based on site-specific data in the formulation and processing stages, the PEC/PNEC ratio is 0.02 or less. Furthermore, the highest available monitoring data from agricultural soil is 5 mg/kg dwt in soil (after application for 10 years with 333 tonnes sludge per year). This indicates that the calculated PECs may be overestimated.

The ESD on Plastics additives suggests that spread-coating sites without air-cleaning release ten times more than an otherwise equivalent site with air-cleaning. Half of the release is supposed to go to waste water. The site specific information from the two spread coating sites (F1 and S8) indicates that the emissions to waste water from spread coating are much lower than what the ESD suggests.

For sealants/adhesives formulation the situation is similar. The site specific information (site F10) indicates much lower emissions to waste water than suggested by the generic release estimation based on default emission factors. The site specific risk characterisation gives a PEC/PNEC ratio below 0.005, which is three orders of magnitude lower than for the generic local scenario (3a sealants formulation).

However, site-specific data is limited to a few out of many sites (in the order of 20 out of 800). The volume used by these sites covers only a few percent of the total volume used in these use areas. The data can therefore not be taken as representative for all other sites (see also Section 3.1.1.2.1). As a realistic worst case it has been assumed that there are plastisol spread coating and sites formulating lacquers, paints, printing inks, sealant and/or adhesives that have emissions of a magnitude as indicated by the rather recently agreed ESD, thus leading to PEC/PNEC ratios > 1.

The PNEC is based on a > NOEC value from a study where no effects on seed germination and growth of plants were seen at the highest test concentration of 100 mg/kg dwt. Studies on other organisms indicate even higher no effect levels. In a 14 days study on earthworms no effects were seen at 1,000 mg/kg dwt and the collembolan species *Folsomia fimetaria* was unaffected at 5,000 mg DEHP/kg dwt after 21 days exposure. The actual PNEC may thus be higher. Repeating the tests at higher test substance concentrations than those used may refine the PNEC. This option is not chosen because data is already available for DEHP and because of the difficulties

associated with testing very high concentrations of a substance. Alternatively more data on emissions and concentrations in soil could be collected. Since a refinement of the exposure assessment may result in a **conclusion (ii)** for this compartment further information could be requested. However since the same scenarios that have PEC/PNEC ratios > 1 for soil organisms, also have PEC/PNEC ratios > 1 for the food chains based on terrestrial organisms, the risk reduction strategy will have to address the emissions in these scenarios anyway. Further studies are therefore not requested at this point.

Therefore, for these use areas **conclusion (i)** "on hold" is drawn.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for the other environmental spheres will eliminate the need for further information on soil organisms.

Urban/industrial soil

The main source for the contamination of urban/industrial soil is the waste formed during use of end-products and "waste remaining in the environment". The distribution of such waste is assumed to be limited geographically (e.g. car undercoating material emitted to soil near roads). A worst case local PEC is therefore expected to be higher than a regional PEC. There are, however, no local scenarios available for the urban/ind. soil compartment in TGD. Only a regional PEC for this compartment has been calculated.

The PEC/PNEC ratios are presented in **Table 3.89**. The regional PEC/PNEC is calculated to be < 0.25. Since worst case local concentrations are expected to be higher than the regional PEC, the PEC/PNEC ratio for a local scenario may be higher than 1.

Table 3.89 Comparison of PECregional for urban/industrial soil with PNECsoil (EUSES)

Scenario	PEC (mg/kg dwt)	PEC PNEC[1]
Regional (EUSES calc.)	3.2	< 0.25
Continental (EUSES calc.)	0.33	< 0.03

1) PNEC = >13 mg/kg dwt

3.3.4 Secondary poisoning

The $PNEC_{oral}$ for DEHP in fresh food is 3.3 mg/kg for mammalians, 17 mg/kg for birds, and 16 mg/kg for fish (see Sections 3.2.4.2 and 3.2.1.6). In this risk assessment we propose that mammalians eat fish, birds eat mussels, and fish eat invertebrates in food chains based on aquatic exposure of DEHP. In the terrestrial food chain mammalians are supposed to eat DEHP exposed earthworms. The PEC/PNEC ratios are presented in **Table 3.90** and **Table 3.91**.

Food chains based on aquatic organisms

The PECorals are calculated based on PECregional surface water (0.8 µg/l) derived from measured data (see Section 3.1.6.1.1). This is considered to give more realistic PEC_{orals} than using the PECregional surface water (2.2µg/l) estimated by EUSES.

At least for fish and invertebrates, BCF values decreases with increasing concentrations of DEHP in water. For example, for fish the BCF is about 800 at the water solubility, 360 at 14 µg/l and 160 at 60 µg/l. Therefore, the water solubility 3 µg/l is used as a limit for the calculation of $PEC_{oral\ aquatic\ fish,}$ i.e. when a $PEC_{surface\ water}$ exceeds the water solubility the water solubility is used for calculating $PEC_{oral\ aquatic.}$ The same approach is used for the calculation of $PEC_{oral\ aquatic\ invertebrates}$ using a wet weight BCF of 2,700 (see Section 3.1.1.4.1). This approach may underestimate the concentration in biota in highly contaminated areas since it can be assumed that in such cases the absorption of DEHP from food becomes increasingly important. However, the highest measured concentration in fish from an extensive study in Austria (Pfannhauser et al. 1997, see Section 3.1.6.5) was 2.6 mg/kg WWT. This value compares quite well with the concentration derived when multiplying BCF with water solubility ($840 \cdot 3.0\ \mu g/l \cdot 10^{-3} = 2.52$ mg/kg WWT).

The available BCF data for mussels does not indicate a negative relation between BCF and DEHP concentrations in water. However, the BCFs may overestimate the bioaccumulation potential of DEHP in mussels since they are based on ^{14}C-activity and not on DEHP and metabolites (i.e. MEHP). Some of the radioactivity measured in the mussels may be due to e.g. $^{14}CO_2$ assimilated by algae and subsequently ingested by the mussels.

Table 3.90 Comparison of calculated PEC_{oral} in aquatic biota with $PNEC_{oral}$, for mammals eating fish, birds eating mussels and fish that eat zooplankton

LOCAL		PEC surf.w[1] (µg/l)	PECoral[2] (mg/kg wwt)				PEC / PNEC[3]	
	prey-->		fish	mussel	invertebrates	fish	mussel	invertebrates
life c.stage	predator-->					mammal	bird	fish
	BCF-->		840	2,500	2,700			
1a Production								
Production site 1		0.8	0.7	2	2	0.21	0.1	0.15
Production site 2		1.8	1.1	3	5	0.32	0.2	0.3
Production site 3		0.8	0.7	2	2	0.21	0.1	0.15
Production site 4		0.9	0.7	2	2	0.21	0.1	0.15
Production site 5		0.8	0.7	2	2	0.20	0,1	0.11
Production site 6		20.4	1.6	27	8	0.48	1.6	0.53
Production site 7		0.8	0.7	2	2	0.20	0.1	0.15
Production site 8		1.2	0.8	3	3	0.26	0.1	0.19
Production site 9		0.8	0.7	2	2	0.21	0.1	0.15
Production site 10		0.8	0.7	2	2	0.20	0.1	0.15
Production site 11		8.2	1.6	11	8	0.48	0.6	0.53
Production site 12		180	1.6	226	8	0.48	13	0.53

Table 3.90 continued overleaf

Table 3.90 continued Comparison of calculated PEC_{oral} in aquatic biota with $PNEC_{oral}$, for mammals eating fish, birds eating mussels and fish that eat zooplankton

LOCAL		PEC surf.w[1] (µg/l)	PECoral[2] (mg/kg wwt)				PEC / PNEC[3]	
	prey-->		**fish**	**mussel**	**invertebrates**	**fish**	**mussel**	**invertebrates**
life c.stage	**predator-->**					**mammal**	**bird**	**fish**
	BCF-->		**840**	**2,500**	**2,700**			
2 Polymer formulation/processing								
2a Polymer-Calendering – generic		8.2	1.6	11	8	0.48	0.65	0.53
	site F3	0.9	0.7	2	2	0.21	0.1	0.15
	site S4	0.8	0.7	2	2	0.21	0.1	0.15
	site S6	1.0	0.8	2	3	0.23	0.1	0.19
2b Polymer-Extrusion comp – generic		3.6	1.6	5	8	0.48	0.3	0.53
	site S5	0.8	0.7	2	2	0.21	0.1	0.15
2c Polymer-Extrusion prod – generic		3.6	1.6	5	8	0.48	0.3	0.53
	site F2	1.0	0.8	2	3	0.24	0.1	0.19
	site F7	0.9	0.7	2	2	0.21	0.1	0.15
	site I9	1.0	0.8	2	3	0.24	0.1	0.19
	site S11	0.8	0.7	2	2	0.21	0.1	0.15
2d Polymer - Plastisol spread coating	with air cleaning - generic	4.2	1.6	6	8	0.48	0.4	0.53
	without air cleaning - generic	29.4	1.6	38	8	0.48	2.2	0.53
	site F1	0.8	0.7	2	2	0.21	0.1	0.15
	site S8	0.8	0.7	2	2	0.21	0.1	0.15
2e Polymer - Other plastisol	*with air cleaning - generic*	2.6	1.4	4	7	0.42	0.2	0.45
	without air cleaning - generic	16.1	1.6	21	8	0.48	1.2	0.53
2? Not known	site ES12	0.8	0.7	2	2	0.21	0.1	0.15
	site ES13	0.80	0.7	2	2	0.21	0.1	0.15

Table 3.90 continued overleaf

Table 3.90 continued Comparison of calculated PEC_{oral} in aquatic biota with $PNEC_{oral}$, for mammals eating fish, birds eating mussels and fish that eat zooplankton

LOCAL		PEC surf.w[1] (µg/l)	PECoral[2] (mg/kg wwt)				PEC / PNEC[3]	
	prey-->		fish	mussel	invertebrates	fish	mussel	invertebrates
life c.stage	predator-->					mammal	bird	fish
	BCF-->		840	2,500	2,700			
3-6 Non polymer form/proc.								
3a Sealants/adhesives:	formulation – generic	84	1.6	106	8	0.45	6.2	0.53
	adhesives site F10	0.8	0.7	2	2	0.21	0.1	0.15
3b Sealants/adhesives	processing – generic	0.8	0.7	2	2.2	0.15	0.1	0.15
4a Lacquers and paint:	formulation – generic	11.7	1.6	16	8	0.45	0.9	0.53
4b Lacquers and paint:	processing – generic	1.6	1,0	3	4	0.3	0.2	0.26
5a Printing inks	formulation – generic	13.3	1.6	18	8	0.45	1.1	0.53
6 Ceramics	formulation – generic	1.1	0.8	2.3	3	0.3	0.1	0.19
7 Municipal STP – generic		3.0	1.6	4.8	8	0.45	0.3	0.53
8a Paper recycling – generic		2.8	1.5	4.5	7.6	0.45	0.3	0.49
8b Disposal - Car shredding – generic		0.8	0.7	2	2.2	0.15	0.1	0.15
8c Disposal - waste incin. – generic		0.8	0.7	2	2.2	0.15	0.1	0.15
REGIONAL		**0.8**	**1.6**	**2**	**2.2**	**0.15**	**0.1**	**0.15**

1) Annual average. Based on PEC regional surface water 0.8 µg/l derived from measured data
2) PECin food is calculated from the assumption that 50% of the diet comes from a local source and 50% from a regional souce. Predator fish are assumed to be stationary and consume all prey locally. Thus, $PEC_{oral\ invertebrates}$ is calculated based only on PEClocal surface water.
3) PNECoral/fish=16 mg/kg wwt; PNECoral/mammal=3.3 mg/kg wwt; PNECoral/bird=17 mg/kg wwt.

Conclusion

For mammalians eating fish, and for fish eating invertebrates the PEC/PNEC ratios are below 1 in all scenarios.

For birds eating mussels the ratio is above 1 for 6 scenarios two being production sites which now have ceased production. For all scenarios based on site-specific reported data in the formulation and processing stages the PEC/PNEC ratio is approximately 0.1. Thus, site specific data indicate that there is no concern for secondary poisoning.

A key parameter for the generic local PEC for plastisol spread coating and other plastisol formulation is whether the site uses exhaust air treatment processes or not. The emission scenario document on Plastics additives suggests that sites without air-cleaning release ten times more

than an otherwise equivalent site with air-cleaning. Half of the release is supposed to go to waste water. The site specific information from the two spread coating sites (F1 and S8) and the two sites (ES12 and ES13) that are assumed to represent other plastisol formulation indicate that the emissions to waste water are much lower than what the ESD suggests.

However, site-specific data is limited to a few out of many sites. The volume used by these sites covers only a few percent of the total volume used in these use areas.The data can therefore not be taken as representative for all other sites (see also Section 3.1.1.2.1). As a realistic worst case it has been assumed that there are plastisol spread coating and sealant/adhesives formulation plants having realeases at levels that are of concern for the sediment compartment.

For adhesives formulation the situation is similar. The site specific information (site F10) indicates much lower emissions to waste water than the generic release estimation based on default emission factors suggests. However, site-specific data is limited to a few out of many sites and the data can therefore not be taken as representative for all other sites (see also Section 3.1.1.2.1). For printing inks formulation no site specific data are available.

Therefore, for these use areas **conclusion (iii)** is drawn.

Food chains based on terrestrial organisms

For mammalians eating earthworms the PEC/PNEC ratios are above 1 for three of the scenarios for formulation and processing of non-polymers and for polymer processing without air cleaning (see **Table 3.91**). For all other local and regional scenarios the ratio is below 1.

Table 3.91 Comparison of calculated PEC_{oral} in terrestrial biota with $PNEC_{oral}$, for mammalians that eat earthworms

life cycle stage	Description		PECoral (mg/kg wwt)	PEC / PNEC[1]
1a	**Production**		**-**	**-**
2	**Polymer formulation/processing**			
2a	Polymer-Calendering – generic		3.5	**1.1**
		site F3	0.05	0.016
		site S4	0.06	0.017
		site S6	0.1	0.031
2b	Polymer-Extrusion comp. - generic		1.3	0.45
		site S5	0.04	0.011
2c	Polymer-Extrusion prod. - generic		1.3	0.45
		site F2	0.14	0.043
		site F7	0.07	0.021
		site I9	0.11	0.033
		site S11	0.04	0.011

Table 3.91 continued overleaf

Table 3.91 continued Comparison of calculated PEC_{oral} in terrestrial biota with $PNEC_{oral}$, for mammalians that eat earthworms

life cycle stage	Description		PECoral (mg/kg wwt)	PEC / PNEC[1]
2	**Polymer formulation/processing**			
2d	Polymer - Plastisol	*with air cleaning - generic*	1.6	0.45
	spread coating	*without air cleaning - generic*	13.4	4.05
		site F1	0.05	0.015
		site S8	0.07	0.020
2e	Polymer -	*with air cleaning – generic*	0.9	0.3
	Other plastisol	*without air cleaning – generic*	7.1	2.1
2?	Not known	site ES12	0.04	0.012
		site ES13	0.05	0.014
3-6	**Non-polymer formulation/processing**			
3a	Sealants/adhesives:	formulation – generic	39	11.6
		adhesives site F10	0.04	0.012
3b		processing – generic	0.04	0.11
4a	Lacquers and paint:	formulation – generic	5.1	1.5
4b		processing – generic	0.4	0.15
5a	Printing ink	formulation – generic	5.8	1.8
6	Ceramics	formulation – generic	0.2	0.08
7	Municipal STP – generic		1	0.3
8a	Paper recycling – generic		1.2	0.3
8b	Disposal - Car shredder – generic		0.04	0.01
8c	Disposal - waste incin. –generic		0.04	0.01
Regional			**0.07**	**0.02**

1) PNECoral/mammal=3.3 mg/kg wwt (based on factors from 2nd edition of TGD)

Conclusion

Available monitoring data indicate that the DEHP concentrations in soil calculated by EUSES may be overestimated (see Section 3.3.3.1). Consequently the concentrations in earthworms may also be overestimated. All site specific PEC/PNEC ratios are far below 1 the highest being 0.043. Thus, site specific data indicate that there is no concern for secondary poisoning of terrestrial predators. The coverage of the site specific data is however low and the representativity is not possible to judge and for the printing inks scenario there are no reported site specific data available (see also Section 3.1.1.2.1). Therefore, on the base of the generic risk characterisation **conclusion (iii)** is drawn for Plastisol spread coating without air treatment, Other plastisol without air treatment, sealant and adhesives formulation and printing ink formulation.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

In summary, exposure to DEHP is expected to occur for the following populations:

Workers (occupational exposure);

- *Production of DEHP*
- Industrial use of DEHP as an additive at:
    - formulation of polymer compound (semi-manufactured products, "dry-blend" and plastisol)
    - formulation of non-polymer
    - processing of PVC polymer products containing DEHP
    - processing of non-PVC polymer products containing DEHP
    - processing of non-polymer products containing DEHP
- Industrial end-use of semi-manufactured products and end-products containing DEHP

Consumers

- Consumer end-use of products containing DEHP
- Via "food contact materials" including food processing and food packaging materials
- During treatment with medical equipment containing DEHP

Humans exposed indirectly via the environment

DEHP has been used to produce flexible plastics that are part of many products intended for both industrial and consumer use. These include building products (insulation of cables and wires, tubes and profiles, flooring, wallpapers, out-door wall- and roof covering, pastes for sealings and isolation mass), children's products (teething rings, squeeze toys, crib bumpers etc.), clothing (footwear, outwear and rainwear), car products (e.g. car under-coating, car seats made of imitation leather) etc. DEHP is also used in non-polymer materials such as lacquers and paints, adhesives, fillers and printing inks etc. An overview of the uses of DEHP is given in **Table 2.3** in Section 2.

Release of DEHP may occur during the production, distribution and incorporation into e.g. PVC-resin. In addition, because DEHP in flexible PVC and other materials are not chemically bound DEHP can be released when the device is heated or can leach out when the device comes into contact with certain media. Consequently, DEHP may be lost from the end-product during its use or after disposal of the product. Leaching may vary widely between products manufactured by different manufacturers or even within the same batch.

Humans may be exposed to DEHP by inhalation of gas, vapour, aerosol and airborne particles containing DEHP.

DEHP is an oily liquid at room temperature with a low vapour pressure (0.000034 Pa at 20°C) and a calculated saturated gas concentration of 5.3 μg/m^3 at 20°C. DEHP may decompose at the temperatures used in PVC processing; however, no data to substantiate this have been found. When DEHP is heated, the vapour pressure will increase with a concomitant increase in the saturated gas concentration. Increased temperature increases the volatilisation of DEHP. The gas will condense at lower temperatures to form a mist (aerosol) or condense on different surfaces e.g. on particles (dust) which might be airborne, and surfaces like walls and windows.

All situations of inhalation exposure to DEHP is a combination of exposure to gasous DEHP, smaller and larger aerosol particles and particles with condensed DEHP on the surface.

The particle size in different environments may be important, both for local effects in the respiratory tract and for the absorption via the lung, or following clearance in the respiratory tract, exposure via the gastrointestinal tract.

DEHP may be airborne as an aerosol if it is a component in a mixture which is sprayed e.g. laquers and paints.

In conclusion, inhalation of DEHP can occur as inhalation of:

- the neat substance as gas or an aerosol of condensed DEHP. A mist may comprise of very small particles with e.g. mass median diameter 0.1-0.3 μm. This kind of aerosol is generated at processed with higher temperatures, where the substance is volitilised and then condense in the air. This is the case at the production and at most industrial uses.
- DEHP as an component in a spray mist of neat DEHP or a mixture of substances e.g in a paint. In general this particles can be assumed to be 2-20 μm, depending on the application tecnique.
- DEHP condensed on other airborne particles

The human population can be exposed to DEHP by different portals of entry: inhalation and oral, dermal and intravenous routes. Based on information contained in Chapter 1 and 2 the following exposure routes for each exposed population are considered to be relevant for this assessment:

- Production via inhalation and dermal routes
- Industrial use via inhalation and dermal routes
- Industrial end-use via inhalation and dermal routes
- Consumer (private) end-use via inhalation, dermal and oral routes
- Medical treatment via intravenous and dermal routes
- Via the environment via inhalation (air) and oral routes (food and water)

Intravenous exposure may occur during medical treatment using articles containing DEHP e.g. blood-bags and dialysis equipment. This exposure, via the intravenous route may be high in some instances. Also it has to be considered that the population may be vulnerable because of ill-health and/or age

In this assessment, all identified sources of human exposure data on DEHP are alluded to. However, there may be other sources of DEHP not yet identified or that are not possible to quantify in terms of exposure.

Bioavailability

Based on toxicokinetic data and information on human exposure situations the percentage bioavailability for different pathways of exposure have been derived and are used in the calculation of internal human exposure. These are summarised:

Summary of exposure route dependent systemic bioavailability

Human exposure route	Human systemic bioavailability (%)
Oral	
Adults	50
Infants/children	100
Inhalation	
Adults	75
Infants/children	100
Dermal (free DEHP and in products)	
Adults	5
Infants/children	5
Parential routes	
All subpopulations	100

4.1.1.1 Occupational exposure

Information in Chapter 2 details important life-cycle steps for occupational exposure. Workers may be exposed to DEHP in the working environment:

- Production of DEHP
- Industrial use of DEHP as an additive at:
    - formulation of polymer compound (semi-manufactured products, “dry-blend” and plastisol)
    - formulation of non-polymer
    - processing of PVC polymer products containing DEHP
    - processing of non-PVC polymer products containing DEHP
    - processing of non-polymer products containing DEHP
- Industrial end-use of semi-manufactured products and end-products containing DEHP

Each occupational exposure is assessed using the available information on the substance (physical and chemical data), processes and work tasks. More detailed information on these

parameters may lead to a more accurate exposure assessment. The exposure is assessed without taking account of the possible influence of personal protective equipment (PPE). Information of the effectiveness of PPE to reduce exposure to DEHP in practical situations ís limited. The use of PPE normally reduce the level of exposure. PPE are usually intended for use during work operations entailing risk for increased exposure such as repair work, service and maintenance.

Information on the process and measured data have only been provided for the production of DEHP and for formulation and processing of PVC polymer products. The process and details of working activities and levels of DEHP in the workplace air during other processes using DEHP may be similar to that for the formulation and processing of PVC polymers; however, there is no information to support or refute this conjecture.

It is envisaged that the work practices for the end-use of semi-manufactured products and end-products by professionals may be activities resulting in occupational exposure.

Data which can be used for occupational exposure assessments are:

- physico-chemical data of DEHP, physical appearance, vapour pressure at different temperatures (see Section 1);
- data regarding methods and use pattern of the product;
- temperature at which production processes take place; amount of DEHP used in the different products (see Section 2).;
- exposure data from HEDSET;
- measured work place data from use of DEHP or other similar phthalates;
- results from exposure models (EASE). [6]

The main routes of occupational exposure are anticipated to be by inhalation of DEHP-gas and liquid aerosol, and by dermal uptake of liquid DEHP, vapour and aerosol. In the polymer industry exposed workers may be working close to processes emitting DEHP, drumming the substance, handling products containing the substance or transferring the substance or the products to other systems.

Much of the gas emitted from the hot processes with DEHP, will rapidly condense to form an aerosol with the consequence that workers will be exposed to both gas and aerosol.

Measured exposure data provide only details on the levels of DEHP present in the working air during production and formulation/processing of PVC polymers and do not examine exposure during other occupations detailed above. Data on biological monitoring, including uptake and effect, and health surveillance have not been provided for the occupations identified.

No data is available on the number of workers in Europe exposed to DEHP. As manufacturing and use of flexible PVC-products is extensive, the number of workers exposed to DEHP could be equally extensive. Due to data from industry (BASF AG, 1999) DEHP is formulated in about 560 sites in EU. The number of sites processing materials containing DEHP is assumed to be

[6] EASE is a general purpose predictive model for assessing workplace exposure. The model is in widespread use across the European Union for the occupational exposure assessment of new and existing substances. EASE is based upon assumptions and the generated data are only approximate. EASE is only intended to generate exposure ranges and works best in exposure assessment when the relevance of the modelled data can be evaluated against measured data. Validation of the information generated by EASE can only be established when compared with reliable measured data.

more than 1,000. In the USA approximately 340,790 employees at 19,400 facilities may be exposed to DEHP (US EPA, 1996).

No information is available on gender or age of the exposed workers in the EU.

4.1.1.1.1 Occupational exposure from production of DEHP

There are at least 15 DEHP production sites in the EU. The production process is described in Section 2.

The production of DEHP takes place in closed systems. However, both inhalation and dermal exposure may occur during the production of DEHP. Such exposure may occur during system leaks (breathing of a closed system), drumming and filling of road- and rail tankers, cleaning e.g. of the tanks in which DEHP has been produced, stored or transported, service and maintenance, transfer, and process sampling.

Inhalation exposure

Measured exposure data of DEHP in the workplace air during production

Exposure data regarding production of DEHP and other phthalates are listed in **Table 4.1**. Measurements were carried out during different work tasks at a number of production sites. Not all reported data include information on e.g. methods for sampling and chemical analysis used, the duration of measurements or task of workers, date when samples were collected or the type of sampling conducted (personal or area measurements).

Table 4.1 Workplace air monitoring of DEHP during production

Industries and tasks	Number of plants	Number of samples	Measured data (mg/m³), full shift		Reference
			Range	Average	
USA					
Production, maintenance (repair of DEHP esterifier), chemical operators	1	50	0.02 - 4.1	0.07	(Liss G.M et al., 19951985)
Europe					
Not specified	1	4	< 0.016-4.3	< 1.09	(King D.A. 1996.), producer 1
Tanker filling	,,	2	< 0.013-0.09	< 0.11	,,
Drumming	,,	1	0.14	-	,,
Not specified	1	1	< 0.1	-	(King D.A. 1996), producer 3
Not specified	1	28**	0.03-1.56	0.36	(King D.A. 1996), producer 2
Not specified	Nd	77	< 5.0	nd	(King D.A. 1996) (HSE data)
Drumming, stationary	1	nd	0.23-0.52	0.32	(Hüls, 1996)
Plasticiser plant, routine. 8 h TWA, personal sampling	,,	38	< 0.05-1.2	median ca. 0.18	,,
Plasticiser plant, short term	,,	12	< 0.1-1.6	median ca. 0.6	,,

Table 4.1 continued overleaf

Table 4.1 continued Workplace air monitoring of DEHP during production

Industries and tasks	Number of plants	Number of samples	Measured data (mg/m³), full shift		Reference
			Range	Average	
Europe					
Personnel at normal running of the process	1	8	< =0.013		Lillienberg, 1999
Laboratory personnel	1	2	< 0.1		,,
Maintenance	1	3	0.07 - 0.14		,,
Shipping	1	2	< 0.08		,,
Stationary sampling, control room and laboratory	1	8	< 0.01		,,
Stationary sampling, reactor sampling site	1	9	0.028 – 0.039		,,
Stationary sampling, process hall	1	9	0.027-0.037		,,
Stationary sampling, process hall	1	10	0.021-0.036		,,
Full shift exposures	1	11	< 0.1		BP Chemicals, 1999
Short term sampling during pure tank sampling, reactor sampling after stripping, SMF sample, reactor sampling, esterification running sample, decanters	1	8	< 0.6		,,
Static samples within laboratory	1	3	0.1		,,

Model generated data for inhalation exposure to DEHP during production

Ranges for inhalation exposure determined with the EASE-model are given below. It is assumed that exposure occurs because of system leaks (breathing of a closed system), with process temperatures up to 250ºC and 20ºC for drumming and cleaning/maintenance. It is expected that the drumming is performed at 20ºC in the presence of Local Exhaust Ventilation (LEV), although the absence of LEV cannot be excluded. Based on this model the estimates of exposure levels of DEHP are the following:

- system leaks; non dispersive use and LEV:
  0.5-3 ppm (8 - 49 mg/ m^3)
- drumming; non-dispersive use with direct handling and dilution ventilation:
  0 - 0.1 ppm (0 - 1.6 mg/m^3)
- cleaning/maintenance; wide dispersive use with direct handling:
  0 - 0.1 ppm (0 - 1.6 mg/m^3)

The measured values from production of DEHP are in the same magnitude as the results from the model calculations.

Measured dermal exposure data during production of DEHP

No measured data on dermal exposure during the production of DEHP have been provided.

Model generated dermal exposure data during production of DEHP

The dermal exposure estimates derived with the EASE-model are given below.

Drumming concerns non-dispersive use with direct handling and intermittent contact, giving an exposure of 0.1-1 mg/cm^2/day. During drumming the palm of both hands may be exposed; this corresponds to an exposed area of 420 cm^2 (approximately half of two hands), which results in a dermal exposure of:

42-420 mg/day

Cleaning and maintenance: it is assumed that the system is flushed with a solvent before opening and that this reduces the concentration of the substance 90% (default value). Non-dispersive use with direct handling and an extensive contact is assumed, giving an exposure of 1-5 mg/cm^2/day. This will be reduced to 0.1–0.5 mg/cm^2/day because of flushing. Assuming that both hands and a part of the forearms are exposed (1,300 cm^2) the dermal exposure is estimated to be 650 mg/day. This exposure will probably not occur frequently but possible up to 10% of the working day.

Use of a transfer line indicates non-dispersive use with direct handling and intermittent contact and gives an exposure of 0.1-1 mg/cm^2/day. It is assumed that the fingers of both hands are exposed. This corresponds to an exposed area of 400 cm^2, which leads to an estimated exposure of:

40-400 mg/day.

Conclusion

The highest measured values, of concentrations in the air, for the production of DEHP are in the same magnitude as the lower values from the model calculations with the EASE-model. Considering all available data for exposure during production of DEHP, a reasonable worst case for exposure via inhalation is estimated to be 5 mg/ m^3 (aerosol) (8-hour TWA) and for dermal exposure to be at 650 mg/day on a skin area of 1,300 cm^2. No measured data for dermal exposure are available.

4.1.1.1.2 Occupational exposure from industrial use of DEHP

Following production, DEHP is incorporated into a polymer or to other mixtures by formulation, see Section 2.4.2.1. There are two major processes for plasticised PVC formulation “dry blending” and “plastisol blending”: The formulated plasticised PVC can then be processed in a number of ways: calendering, extrusion, injection moulding, several plastisol applications including rotational moulding, dip coating, slush moulding, spray coating and miscellaneous small to very small applications.

The processing of the “dry-blended”-formulations is performed by heating the blend in one or several stages e.g. by friction or by heating the surfaces and transferring it into the molten state. The moulding occurs above 160°C. The major processes for “dry-blend” are calendering, extrusion and injection moulding

The "plastisol-blend" is applied by spreading on a substrate (e.g. paper or fabric) and thereafter heated (= "gelled" or "fused") to typically above 160°C. Major application modes for plastisol are spreading (spread coating). dipping of moulds into plastisol. spraying or injection of pseudoplastic onto car under-coating and slush - rotational moulding

Based on this information, the following industrial uses of DEHP are identified:

- formulation of polymer compound (semi-manufactured products, "dry-blend" and plastisol)
- formulation of non-polymer
- processing of PVC polymer products containing DEHP
- processing of non-PVC polymer products containing DEHP
- processing of non-polymer products containing DEHP

The handling of DEHP in the polymer industry (formulation/processing) may more often be open processes than during production. Information has been submitted by industry only on the formulation/processing of PVC polymer products. This information includes details on workplace measurement data and process and detail description.

Formulation and processing of polymer products containing DEHP

Exposure may occur in the following situations during the formulation and processing of flexible PVC;

- adding (compounding)
- mixing the agent
- forming into shapes
- service and maintenance

Much of the gas emitted from the hot plasticised PVC will rapidly condense to form an aerosol with the consequence that workers will be exposed to both gas and aerosol.

Inhalation exposure

Measured data of DEHP in the workplace air during formulation/processing

Although DEHP is extensively used, few measured data on exposure to DEHP are available. Data which are available are not, however, considered to be of sufficient quality because: 1) the reported information is inadequately documented, for instance, it is not detailed whether exposure to gas or aerosol was measured; and, 2) the data are not judged to be reliable because the data are not considered representative of the overall exposure within the EU nor may they represent the present day situation.

Exposure data regarding formulation and processing of PVC products containing DEHP and other phthalates are available. Measurements were carried out at different areas of a number of production sites. Not all reported data include relevant detail regarding e.g. methods for sampling and chemical analysis, number of measurements, duration of measurements or task of workers. Relevant information from measurements are presented in **Table 4.2**.

Table 4.2 Measured inhalation exposure to phthalates mainly DEHP during the formulation/processing -plasticised PVC.

Substance	Activities	Process temperature (°C)	Country	No. of plants	No. of samples	Conc. (mg/m³), full shift		Duration (h)	Ref.
						Range	Average		
DEHP	Extrusion	150-200	FIN	**	4		0.05±0.03	1.5-3	(Vainiotalo, 1990)
	Extrusion	150-195		**	5		0.3±0.2	1.5-3	”
	Calendering	130-200		**	7		0.5±0.5	1.5-3	”
	Hot embossing	≈180		**	5		0.05±0.02	1.5-3	”
	Welding	400		**	4		0.3±0.05	1.5-3	”
	Injection moulding	180-190		**	2		0.02±0.01	1.5-3	”
	Compounding	120		**	5		0.02±0.01	1.5-3	”
	Thermoforming	120-130		**	2		0.02±0.02	1.5-3	”
	High-frequency welding	nd		**	N		<0.02	1.5-3	”
DEHP	Manufacture of pigment dispersions	nd	UK	nd	8	<0.25		Nd	(HSE, 1984)
DEHP	Recovery of filter residues	nd		nd	11	<1.0		Nd	”
DEHP	Manufacture of floor tiles	nd		nd	8	<0.5		Nd	”
DEHP, BBP[3]	Manufacture of flexible floor covering	nd		nd	12	<0.5		Nd	”
DEHP	Manufacture of PVC	nd		nd	7	<0.5		Nd	”
Various, mainly	Calendar operators		SE	1	12	1.0-2.8	2.0	2	(Nielsen et al, 1985)
DEHP; DIDP and BBP	Calendar operators/machine attendants			1	16	0.1-0.8	0.4	2	(Nielsen et al, 1985)

Table 4.2 continued overleaf

Table 4.2 continued Measured inhalation exposure to phthalates mainly DEHP during the formulation/processing -plasticised PVC

Substance	Activities	Process temperature (°C)	Country	No. of plants	No. of samples	Conc. (mg/m³), full shift		Duration (h)	Ref.
						Range	Average		
	Machine attendants			1	8	0.1-0.2	0.2	2	(Nielsen et al, 1985)
	Repair men			1	8	0.1-0.3	0.3	2	(Nielsen et al, 1985)
	Mixing workers			1	8	0.01-0.02	0.02	2	(Nielsen et al, 1985)
	Others			1	44	0.1-0.3	0.1	2	(Nielsen et al, 1985)
Various, mainly	Calendar operations	nd	SE	1	Nd	0.5-3*			(Hagmar et al, 1990)
DEHP; DIDP and BBP	Working in mixing departments and machine attendants	nd		1	Nd	0.1-0.5*			”
	Quality inspectors and packaging personnel	nd		1	Nd	up to 0.1*			”
DEHP	Mixing, boot factory		NL		16	0.1 –1.2	0.26	2	(Dirven H.A.A.M. et al, 1993)
	Extruder, boot factory				11	0.05-0.3	0.12	2	”
	Mixing, cable factory				8	0.01-0.8	0.18	2	”
	Extruder, cable factory				13	0.01-1.3	0.23	3	”
	Extrusion, injection moulding, calendering, compounding		NL		34	0.02–0.5			RIVM, 1997
	Calendering		NL		3	1.46-1.95			”
	Calendering		NL		6	0.3-2			”
	Waste processing		NL		1	1.23			”
	Rubber (calendering)		FR		25	0.04–26.7	2.48±,5.98		INRS, 1997***

Table 4.2 continued overleaf

Table 4.2 continued Measured inhalation exposure to phthalates mainly DEHP during the formulation/processing -plasticised PVC

Substance	Activities	Process temperature (°C)	Country	No. of plants	No. of samples	Conc. (mg/m³), full shift		Duration (h)	Ref.
						Range	Average		
	Pharmaceuticals		FR		10	0.03-1.55	0.28±,0.54		”
	Metallic hoses		FR		8	0.0007-0.07	0.016±0.023		”
	Application of car sealings and car under-coating				3	< 0.001 - 0.11			Hüls, 1995#

1) The two studies (Nielsen et al, 1985) and (Hagmar et al., 1990) are performed at the same factory.
* No information if personal or area measurements
** Total samples collected from nine different factories
*** Dioctyl phthalate exposure measurements recorded from 1987 to 1996 in the COLCHIC database
nd no data,
DIDP di-isodecylphthalate,
BBP benzylbutylphthalate

The very high concentrations of DEHP found in the COLCHIC database for the rubber industry apply to the calendering of a rubber containing 4-8% dioctyl phthalate at more than 200°C, at a speed of 12 m/min. The measurements were performed in the ambient atmospheres, in places where workers were only present in case of problems or to check processing conditions. The highest measured concentration occurred for a sampling time of 3 hours. If there is no additional exposures the 8-hour TWA is in this case 10 mg/m^3. These data indicate, however, that short-term concentrations could be as high as 30 mg/m^3.

Measured occupational DEHP exposure data (8-hour time weighted average) gathered during calendering, extruding, gluing and welding of plastics in Germany collected from 1991 to 1995, are listed in **Table 4.3** (BGAA, 1996). The results show higher exposure at working areas with "ventilation" than at working area without "ventilation". This difference probably reflects different working activities at different working areas in the presence and absence of ventilation i.e. ventilation is used in areas of high exposure. However, there is no information on the efficiency of the use of ventilation equipment at different working sites which may be very different from each other.

Table 4.3 Measured occupational DEHP exposure data (8-hour time weighted average) gathered during calendering, extruding, gluing and welding of plastics in Germany collected from 1991 to 1995 (BGAA, 1996)

Type of company/work area	Number of measurement data	Number of companies	50%-value (mg/m^3)	90%-value (mg/m^3)	95%-value (mg/m^3)
without ventilation	32	14	0.03	0.44	0.57
with ventilation	53	21	0.15	3.65	7.00
Total	**85**	**31**	**0.08**	**2.45**	**5.93**

Model generated data for inhalation exposure to DEHP during formulation/processing

The inhalation exposure estimates derived with the EASE-model are given below:

- transition of DEHP into a mixer at room temperature; non-dispersive use and LEV: 0-0.1 ppm (0 -1.6 mg/m^3);
- adding of DEHP to mixers at 20ºC: Non-dispersive use, direct handling, dilution ventilation: 0-0.1 ppm (0 - 1.6 mg/m^3);
- calendering; 200°C, non-dispersive use, LEV present: 0.5 - 3 ppm (8 - 49 mg/m^3).

Measured dermal exposure data during formulation/processing

No data on dermal exposure of DEHP have been provided by the industry.

Model exposure data during formulation/processing

Dermal exposure during formulation and processing of DEHP may occur during transition DEHP into mixers, manual adding of DEHP to mixers, cleaning, service and maintenance of the process equipment, manual handling of semi-manufactured products and end products, and other manual activities.

During most stages of the process, dermal exposure is not expected to occur, because closed-system process is used separated at high temperature.

Based on the EASE-model the estimates of the potential dermal exposure level of DEHP are the following

Transition: non-dispersive use with direct handling and incidental contact: 0 - 0.1 mg/cm^2/day, all fingers may be exposed, the exposed area is estimated to be about 400 cm^2, giving an exposure of 0-40 mg/day.

Manual adding of DEHP to mixers: non-dispersive use with direct handling and intermittent contact: 0.1-1 mg/ cm^2 /day, assuming that one hand can be totally exposed, the exposed area is estimated as about 420 cm^2 and results in an exposure of 42 - 420 mg/day.

Cleaning, service and maintenance: non-dispersive use with direct handling and incidental contact: 0 - 0.1 mg/cm^2/day. It is assumed that both hands and a part of the forearms will be exposed. The exposed area will be 1,300 cm^2. The exposure will be 0 - 130 mg/day.

4.1.1.1.3 Formulation and processing of non-PVC polymer products containing DEHP

Individuals may be exposed to DEHP during formulation and processing of non-PVC polymer products containing DEHP.

No measured exposure data on the inhalation exposure to DEHP in workplaces during formulation and processing of non-PVC polymer products, have been submitted.

The available information on formulation and processing of non-PVC polymer products is insufficient to estimate exposure range with EASE.

4.1.1.1.4 Formulation and processing of non-polymer products containing DEHP

Individuals may be exposed to DEHP during formulation and processing of non-polymer products containing DEHP.

No measured exposure data on the inhalation exposure to DEHP in workplaces at formulation and processing of non- polymer products, have been submitted.

The available information on formulation and processing of non-polymer products is insufficient to estimate the inhalation and dermal exposure range with EASE.

Summary/statement of the exposure level

The highest measured values, of concentrations in the air, for the industrial use of DEHP are in the same magnitude as the lower values from the model calculations with the EASE-model. Because of the large number of sites within this group in the EU, and that we assume it is an inhomogenous group in the respect of size of the plants, processing techniques, ventilation equipment's etc, we assume, a reasonable worst case for exposure via inhalation is estimated to be 10 mg/ m^3 (8-hour TWA) and for dermal exposure to be at 420 mg/day on a skin area of 420 cm^2. No measured data for dermal exposure are available.

4.1.1.1.5 Industrial end-use of products containing DEHP

Few measured exposure data of DEHP in the workplace air at industrial end-uses of DEHP have been submitted.The available information on industrial end-use of DEHP is insufficient to estimate the inhalation and dermal exposure range with the EASE-model. No data of the number of sites were industrial end-use of DEHP are taking place are available.

All products intended for industrial use containing DEHP may lead to human exposure. Hence, the extent of exposure may be high and multiple routes of exposure may occur.

The use of polymer materials is very prevalent in many industrial branches and individuals may be exposed to DEHP from the products.

DEHP may be included in PVC or non-PVC products, such as adhesives/sealings lacquers/paints rubber, inks for textiles and ceramics for electronic purpose.

End-products are assumed to emit relative little vapour, since they are generally used at room temperatures. Significant exposures could occur with heated products (e.g. hot-melt adhesives) or with aerosol-forming activities (e.g. textile spread coating, car under-body spray coating); actual DEHP concentrations may however, be limited due to their low vapour pressure, the range of particle sizes generated (they may not be respirable if not formed by a re-condensation mechanism), or their percentage in formulations.

There are very few exposure data available for this scenario. Although exposure is likely to be very low in many circumstances, there is no clear evidence that worst-case exposure during aerosol forming activities would be lower than for the industrial use of DEHP. Therefore worst-case exposure is assumed to be 10 mg/m^3 (8-hour TWA).

Conclusion

Because of the large number of sites within this group in the EU, and that we assume it is an inhomogenous group in the respect of size of the plants, processing techniques, ventilation equipment's etc, we assume, a reasonable worst case for exposure via inhalation is estimated to be 10 mg/ m^3 (8-hour TWA) and for dermal exposure to be at 1,300 mg/day on a skin area of 1,300 cm^2. No measured data for dermal exposure are available.

4.1.1.1.6 Exposure control

Qualitative description of production, formulation and processing of DEHP indicates that both technical and personal protective measures are used. However, reliable documentation to demonstrate the reliability and representativiness of these measured data are not available.

To determine that protective measures maintain DEHP levels at a relatively low level, reliable and representative data are necessary. The available monitoring data are considered inadequate to fulfil this requirement.

4.1.1.2 Occupational exposure -Internal exposure

4.1.1.2.1 Inhalation

Calculation methods

The occupational internal exposure by inhalation can be calculated:

$$U_{inh} = \frac{B_{inh} \times C_{inh} \times V_{inh}}{BW}$$

Values used for the calculation of inhalation exposure to airborne DEHP are as follow:

U is the uptake (mg/kg/day),

B_{inh} the bioavailability for inhalation exposure (75%/100),

C_{inh} the air concentration (mg/m^3),

V_{inh} the inhalation rate (10 m^3/day),

BW the body weight of a worker (70 kg) and

t_{exp} exposure duration (8h/day)

Dermal exposure

The occupational internal exposure by dermal absorption after exposure to DEHP can be calculated, using the following formula;

$$U_{derm} = \frac{B_{derm} \times C_{derm} \times S_{derm}}{BW}$$

Values used for the calculation of exposure to undiluted DEHP are as follow:

U is the estimated total uptake (mg/kg bw/day),

BW the body weight of a worker (70 kg),

S_{derm} the surface area of exposed skin,

C_{derm} is the amount of DEHP per skin area unit and day (mg/cm^2/day) and

B_{derm} is the bioavailability for dermal absorption of the daily external exposure of DEHP (5%/100).

The exposure data for scenarios of external exposure for the occupational exposure scenarios to DEHP used for the calculation of internal exposure are summarised in **Table 4.4**.

Table 4.4 Summary of the exposure for the occupational exposure scenarios of DEHP

Scenario	C_{inh} Concentration of DEHP in the inhaled air (mg/m³)	U_{inh} Calculated internal exposure (µg/kg BW/day)	C_{derm} The amount of DEHP per skin area unit and day (mg/cm²/day)	S_{derm} The skin area exposed (cm²)	Daily dermal exposure (mg/day)	U_{derm} Calculated internal exposure (µg/kg BW/day)
Production of DEHP	5	530	0.5	1,300	650	460
Industrial use of DEHP	10	1,060	1	420	420	300
Industrial end-use of products containing DEHP	10	1,060	1	1,300	1,300	928

4.1.1.3 Consumer exposure

There is no information indicating that DEHP alone is available to consumers, however, DEHP is used in several products, see Section 2.4.1, some of which are available to consumers.

Consumers may be exposed to DEHP released from consumer products. Because plasticisers in flexible PVC and other materials are not chemically bound, they may be released from the finished article during its life-time. Exposure can also occur from several sources by different routes in different situations.

The exposure intensity is expected not to be linear over the product life-time. New products are expected to produce a higher exposure potential than products in which DEHP has reached a steady-state release from the product matrix to medium.

Many DEHP-containing products principally PVC-products, are used outdoors e.g. roofing sheet, coated steel sheet, tarpaulins, automotive undercoating. One would expect little direct human exposure to DEHP from these products. Other PVC products may be used indoors, e.g. cable insulation and window frames, but only a small fraction of their total area would be available to emit DEHP.

The following product groups are considered for consumer exposure to DEHP:

- Toys and child-care articles
- Building materials and home furnishing
- Car interiors
- Clothing (rainwear and clothes of artificial leather), gloves and footwear
- Medical devices
- Food contact materials

Data which (if available) are used for the consumer exposure assessment are actual exposure data, results from mathematical models for consumer exposure and empirical measurements of migration.

The routes of exposure will include inhalation, dermal and oral and possibly combinations of these routes. Some examples of the sources of exposure for different routes are:

- *inhalation* - release of DEHP from building materials (wallpaper, floor coverings etc.), home furnishing, car interiors etc.
- *dermal* - skin contact with footwear, rainwear, PVC gloves, artificial leather on furniture and car seats, toys etc.
- *oral route* - baby- and children's products and food contact materials

4.1.1.3.1 Exposure by food contact materials

In this report, materials which come into contact with food e.g. during processing in the food industry and food packaging are called "food contact materials".

Contamination of food by DEHP can occur during processing, handling, transportation and packaging of food and via "secondary" food storage articles.

During processing, food may be contaminated from PVC tubing and other process equipment containing DEHP. For example, transfer of DEHP from tubes to milk during different operations in dairies may occur (Wildbrett et al., 1977, Castle et al., 1990). DEHP may be used in lubricants in the food processing industry e.g. at slaughter-houses.

Contamination of DEHP in food products can occur via polymer and non-polymer products contained in food packaging material. For instance, DEHP in printing ink used for flexible food packaging, glues used for paper and plastics, in aluminium foil-paper laminates and closure seal in bottles (MAFF, 1996c).

Contamination of DEHP from packaging material is dependent both on the concentration of DEHP in the packaging material, the rate of migration from the packaging material to the food product and the time under which contact occurs. The concentration of DEHP in the food from packaging material may not be evenly distributed as migration within the food i.e. from the surface of contact to the centre is time dependent.

No data on the DEHP contamination in the EU of food caused by food contact material have been submitted by the industry.

The source of DEHP in food is not often known for monitoring data.

No attempt to model DEHP exposure from food contact materials alone are made in this assessment as there are few data available to allow this.

Although never widely used in food contact materials, the use of DEHP has declined during recent years (MAFF, 1996c). However, long term exposure to low doses of DEHP from food contact materials may occur.

Exposure is expected to occur via oral route following ingestion. However, exposure by inhalation may occur from packaging materials containing DEHP for pre-prepared foods intended for direct heating in e.g. micro-wave oven without removal of the packaging material. Additional food contamination may also occur during heating. No measured or modelled data on this exposure is available.

The available measured data of DEHP in foodstuffs are used as a reasonable worst case on consumer exposure via food.

Food and drink may be exposed both via the environment and via non-environmental sources such as food contact materials. Because the media (i.e. food) and population are the same for

convenience, these results are presented in Section 4.1.1.4. "Human exposed via the environment".

4.1.1.3.2 Toys and child-care articles

Many soft plastic toys and teethers are composed of PVC plastic and can contain a high concentration of DEHP. Teethers, commonly used as child-care products or as toys for babies are manufactured especially for chewing/biting by babies at the time when their teeth start erupting.

The Laboratory of the Government Chemist, UK, have 1993 investigated the content of plasticisers in 113 plastic teethers and toys. In 82 samples containing phthalates, 20 had DEHP as the major component. The weight loss after diethyl ether extraction was used as a measure for the plasticiser content and losses of around 50% were quite common. The year when this study was performed is unknown (CSTEE, 1998a).

In an investigation published by Greenpeace 1997, DEHP was analysed in 72 children´s toys purchased in 17 countries: 31 toys were purchased in EU. Of the 72 toys 64 contained PVC. Of the 31 toys, 22 were made of PVC, 5 contained PVC-sections and 4 did not contain PVC. 25 of the 64 (total) or 8 of 27 (EU) toys contained trace amounts of DEHP, less than 1%. This is probably due to the presence of DEHP as an impurity in DINP. In 7 toys, DEHP was used as a plastisiser in concentrations of between 10 and 35%. Two of these samples were bought and made in EU- countries, and five were bought in Asian countries. Of the toys bought in Asian countries, two were made in Japan, one in China and two were of unknown origin. At least half of all the 72 products were produced in China (Stringer R, 1999).

Teethers available on the Spanish market were analysed for DEHP and DINP (di-isononylphthalate) and the two phthalates were detected in 12 out of 30 samples. The year when this study was performed is unknown (CSTEE, 1998a).

The UK Government has followed the content of plasticisers in PVC articles (CSTEE, 1998a). DEHP were found in 4 of 18 articles in 1990, 13 of 27 articles in 1991, 2 of 16 articles in 1992 and 1 of 29 articles in 1996. This study and the results of the Greenpeace (1997) study (2 of 27 samples in 1997) indicates a possible decrease in the use of DEHP in articles

In one study (Rastogi S.C. et al., 1998), 16 toys were analysed for the contents of organic solvents and phthalates. Sub-samples of the three investigated dolls (head and arm made of soft plastic) were found to contain 20-27% di-isononyl phthalate (DINP) or DEHP.

Children may be exposed to DEHP from toys made of plasticised plastic material by the oral and dermal routes. A significant route of exposure for small children when sucking on plasticiced articles is via the mucous membrane in the mouth.

Exposure via inhalation of DEHP-vapour from the toys is probably low.

4.1.1.3.3 Oral exposure

For small children the oral exposure is the predominant exposure route as they suck, chew and bite the toys. The physical "chewing" and at the same time provision of fresh saliva around the article can be a rather effective extraction procedure for DEHP.

Leached amounts

It is difficult to estimate the DEHP exposure of children chewing on toys and teethers made of PVC containing DEHP. The rate of migration is affected by factors such as the relative solubility of DEHP in the PVC-polymer and in saliva, the temperature, the thickness of the polymer and the physical forces acting on the polymer.

The available extraction data are mainly produced in dynamic leak tests and may be underestimates of real life situations.In an Austrian study with adult volunteers, male and female, the release of DEHP and DINP from PVC-sheets was determined (Steiner, 1998). The volunteers chewed or sucked on the PVC-sheets and their saliva was collected and determined for DEHP and DINP. The results were compared with other results from different methods: static method and methods using shaking and ultrasonic extraction. The highest release of DEHP was obtained by the method of sucking.

Two other studies designed to determine the release rates of other phthalates, especially DINP, from PVC samples into saliva have been carried out recently (Könemann 1998, LGC, 1998).

At present, there is, however, no standard method available to mimic the exposure during chewing.

There are a number of determinations of phthalate leachate from toys reported. **Table 4.5** presents the available data on leaching of DEHP from materials on the European market.

Table 4.5 Reported leaching of DEHP from toys under static and dynamic experimental conditions

Leaching of DEHP	Unit	Calculated maximum oral dose* (µg/kg bw/day)	Reference
nd - 4,193	µg/dm^2/24h	13	Vikelsoe et al. (1997)
Nd	mg/kg/6h	-	ARTSANA in CSTEE (1998a)
1,790 - 2,130	µg/dm^2/6h	27	Pindar A et al. in CSTEE (1998a)
30 – 720	µg/cm^2/h	54	Spanish Ministry of Health and Consumer Affairs in CSTEE (1998a)
10.5 - 652.9	µg/dm^2/6h	8.2	CSTEE (1998)
200 – 1,000	µg/dm^2/h	75	Greenpeace (1997)
<4 – 10	µg/dm^2/24h	< 0.03	CEFIC-ECPI (1998) in CSTEE (1998a)
<100	µg/dm^2/h	<8	CEFIC-ECPI (1998) in CSTEE (1998a)
<50 – 180	µg/dm^2/24h	0.56	CEFIC-ECPI (1998) in CSTEE (1998a)
793	µg/dm^2/3h	19.8	Steiner (1998)**
014 - 0.074	µg/ cm^2/h.		Turnbull and Rodricks (1989)

Nd Not detected
** Sucking by voluntary test persons
*** 27 pacifiers, 12 teethers and 18 toys

For calculation of the individual oral exposure to DEHP from these studies values have been used assuming a maximum exposure of 3 hours/day, with a mouthing area of 10 cm^2 for a child with 8 kg body weight.

Information on the exposure from pacifiers is not reported here as DEHP is no longer used in pacifiers in Europe.

Conclusion

The criteria's for calculation of oral exposure in children suggested by the CSTEE are used here. To estimate the internal exposure it is assumed that the bioavailability for DEHP by the oral route for children is 100%. The leached amount of DEHP thus have been calculated per area and time.

Reported leaching of DEHP from toys under static and dynamic experimental conditions, are presented in **Table 4.5**.

Information was achieved from an Austrian study in volunteers on the release of DINP and DEHP into saliva from PVC-sheets and -toys (Steiner, 1998). In a Dutch volunteer study the release of DINP was studied (Könemann, 1998). The mean releases of DINP after sucking found in the Dutch and Austrian studies were similar, 146 and 132 m g/10 cm^2 and hour, respectively. The release of DEHP during sucking (not using the teeth) was investigated in the Austrian study. The result was almost the same as for the corresponding release of DINP in the same type of experiment. CSTEE has therefore assumed that the DEHP release when the toy in addition is chewed, also would have been of the same magnitude as for DINP in the chewing experiment. For DEHP it will use the same maximum release rate as for DINP as a worst-case scenario. This means that daily internal exposure for a child weighing 8 kg, with an daily exposure period of 3 hours and a product surface area of 10 cm^2 would be 200 µg /kg/day. (CSTEE, 1998b)

Dermal exposure

DEHP is used in a variety of products where DEHP can be transferred to the skin via direct physical contact with infants skin. These products include teethers, squeeze toys, crib bumpers and playpen covers. The amount of DEHP that a child is exposed to is a function of the area of skin in contact with the product, the duration of the contact, the surface availability of DEHP from the product and the penetration of DEHP through the skin.

The maximum internal exposure (U_{max}) by dermal contact to toys is calculated using a dermal absorption rate (J) for contact with articles containing DEHP (Deisinger et al, 1998). See model (I);

Model (I)

$$U_{\max} = \frac{J \times S_{derm} \times t_{\exp}}{\mathrm{BW}} = \frac{0.24 \times 100 \times 3}{8} = 9 \text{ µg/kg bw/day}$$

Alternatively, the calculation can account for the migration of DEHP from products (Hudson, 1983) and then the percentage absorption through the skin (5%) which has already been corrected for interspecies differences (see model II below).

Data on the release of DEHP from products in contact with skin is limited. Contact with gums (mouth parts) is not considered.

Experiments designed to model the dermal exposure from contact with products containing DEHP have been performed. A flat vinyl product containing DEHP was scrubbed with a cotton cloth attached to a wood block. Experiments were conducted where the cotton was impregnated with lanolin to represent the oils present on human skin. The DEHP release value from the product analysed is; R = 0.11 µg/cm^2/min (6.6. µg/cm^2/hour) (CMA, 1984). From this data the exposure can be calculated with model (II):

Model (II)

$$C_{derm} = R \times t = 6.6 \times 3 = 19.8\,\mu g / cm^2$$

$$U_{derm} = \frac{B_{derm} \times C_{\text{derm}} \times S_{\text{derm}}}{\text{BW}} = \frac{0.05 \times 19.8 \times 100}{8} = 12.4\,\mu g / kgBW / day$$

For both approaches it is assumed that the the skin contact area S_{derm} = 100 cm^2 (estimated skin area round the mouth and on the hands in contact with the toy), contact duration; t = 3 hours/day, and child's body weight BW = 8 kg.

Comparison of the results derived from these two approaches are in close agreement. The value selected for the risk caracterisation without correction for interspecies differences is that based on the percutaneous adsorption rate derived from Deisinger et al. (1998) i.e Model I.

4.1.1.3.4 Indoor air (building materials)

Many materials used in modern buildings - houses and flats, work-places, public buildings etc., are made of flexible PVC containing DEHP as a plasticiser.

The great majority of flexible PVC is used indoors in applications such as e.g. flooring, wall covering, upholstery, wire and cable. Under normal indoor conditions of temperature and air flow, one should anticipate that DEHP is emitted from these products. Volatilisation in an indoor environment occurs from a combination of chemical diffusion in air and transport as a result of air flow within the room. The mechanism controlling DEHP emission depends on the shape and surface area of the vinyl product, its DEHP content, the air flow across the product, product usage, etc. DEHP in plastic volatilises into the air at a low but detectable rate which, in an indoor environment, result in low levels of inhalation exposure to individuals. In addition, inhalation exposure to DEHP may occur as aerosol of DEHP or DEHP adsorbed to particulate matter. The particle inhalation exposure to DEHP is considered to be significant due to its low vapour pressure. This latter form of exposure has been identified in a Norwegian study that examines residential exposure and considers particulate material both suspended in the air and sedimented in the home (Øie et al.,1997). It is possible that exposure to DEHP in the form of PVC particles may occur from material due to mechanical wear: However, due to Øie et al.(1997), this is not likely to be the case.

In a Danish study (Clausen et al, 1999), semi-volatile organic compounds in air have been sampled from four offices in one building, a classroom in a public school, and a room in a day-care centre in Denmark on one winter day and one spring day. The median concentration for DEHP was 858 ng/m^3.

In a study of phthalates and PAHs in 125 Californian homes (Sheldon et al.,1993) it was found that the daytime (12 hours) air concentration (aerosol + gas phase) of DEHP had a median of 110 ng/m^3 and a 90th percentile 240 ng/m^3.

In a Norwegian study (Øie L. et al.,1997), aerosols have been sampled in six residences and the phthalate content reported as mean values i µg phthalate per 100 mg aerosol. However, calculations of the air concentration give a minimum air concentration of 1.8 ng/m^3. The maximum values are hardly more than 20 times this value. In this study, the phthalate concentrations have been measured in the aerosol fraction sampled on a filter only.

In a German study (v. Pöhner A et al., 1997) semi- and sparingly volatile organic compounds have been measured in dust from dwellings in West-Germany. In 272 samples the maximum concentration of DEHP was 8,600 ppm and the 90th percentile was 1,600 ppm. DEHP has been measured in indoor air in rooms with new flooring (Wams, 1987). The total DEHP concentration was around 0.2-0.3 mg/m^3.

It is difficult to evaluate these data, because limited information on the use of articles containing DEHP or activities in the room etc. is not available.

Personal samples are not available which makes the estimation of the exposure more difficult.

Exposure may be modelled to predict an exposure per event. The level of indoor DEHP in air released from floor and wall covering can be calculated. The concentration of DEHP in the air depends on factors which include, the volume of the room, ventilation rate, temperature, surface area of the plastic article, the amount of DEHP in the plastic material etc. Volatilisation of DEHP based on an emission rate has been calculated by three different methods (see Environ Corporation, 1988). An emission rate of between $1.8 \cdot 10^{-4}$ and $3 \cdot 10^{-4}$ µg/m^2/s at 25°C was derived. The highest emission is used as a worst case in this risk assessment.

The concentration of DEHP released into a room can be derived from a mass balance calculation:

At steady state the concentration (C) can be calculated as follows:

$$C = \frac{3.600 \times E \times A}{ach \times V}$$

Where:

E = DEHP emission rate, $3 \cdot 10^{-4}$ µg/m^2/s

A = area of the PVC material, m^2
ach = air change rate (air changes/hour)
V = volume of the room, m^3

In a room covered with PVC flooring and wall material with the dimension $2 \cdot 3 \cdot 2.4$ (meters: $l \cdot b \cdot h$) the total area covered with PVC material is 30 m^2 and the room volume 14.4 m^3. The air exchange rate is 0.5 ach and the exposure concentration is:

$$C = \frac{3.600 \times 3 \times 10^{-4} \times 30}{0.5 \times 14.4} = 4.5\ \mu g/m^3$$

A study conducted to determine the amount of DEHP bound to particles in residential air in Norway have been reported (Øie et al., 1997).

The vapour phase exposure was not measured but reference to calculated exposure was made. Based upon vapour phase exposure and their measurements, the authors tentatively suggest that exposure via the particulate phase is 1-3 fold greater than exposure from the vapour phase. That 3-fold more DEHP than the vapour concentration can bind to dust particles is supported by the findings that DEHP:

- can migrate from and into different matrices
- that substances with a low vapour pressure are readily adsorbed to particles
- total air concentrations of DEHP has been shown to exceed the saturated vapour pressure by 100-fold (Wams, 1987).

Because there are no data characterising particle-bound DEHP in indoor air, it is considered reasonable to assume that the amount of DEHP associated with particles is three times more than the amount of DEHP present as vapour in the air.

We, therefore, propose that human exposure estimated based the vapour exposure in air and three times this value bound to dust particles avoids underestimation of the amount of DEHP in indoor air.

A concentration of DEHP indoors, at 20°C can be calculated using saturated vapour pressure a concentration of 5.3 μg/m^3. This concentration is in the same magnitude as the calculated concentration above. We assume 5.3 μg/m^3 to be a reasonable worst case. Since the Norwegian study indicates that three times more DEHP is bound to particles, it can be inferred that 15.9 μg/m^3 (3 · 5.3) are adsorbed on to particles. The total air concentration may then be 21.2 μg/m^3.

The size of the suspended airborne dust particles in Norwegian homes is less than 2.5 μm in diameter (Ormstad et al, 1997). The concentration of particles in the air, in the 29 households examined, varied between 9 and 56 μg/m^3.

The daily exposure to DEHP in in-door environments for adults and children is calculated, see **Table 4.6**. The body weight (bw) for an adult is 60 kg and 8 kg for a child. The inhalation rate (V_{inh}) for an adult is 20 m^3/day and 9.3 m^3/day for a child (Whalan et al., 1997).

$$U_{inh} = \frac{B_{inh} \times C_{inh} \times V_{inh} \times t / 24}{BW}$$

Values used for the calculation of inhalation exposure to airborne DEHP are as follow;

U is the uptake (mg/kg/day), B_{inh} the bioavailability for inhalation exposure (75% for adults and 100% for children), C_{inh} the air concentration (mg/m^3), V_{inh} the inhalation rate (20 m^3/day for adults and 9.3 m^3/day for children, BW the body weight (60 kg for adults and 8 kg for children), and t_{exp} exposure duration (20 hours/day for adults and 22 hours/day for children).

$$\text{Adults: } U_{inh} = \frac{0.75 \times 21 \times 20 \times 20 / 24}{60} = 4.4 \text{ μg/kg bw/day}$$

BW = 60 kg, Inhalation rate = 20 m^3/day (adults 30-<60 year, respiratory volume while at rest 372 l/hour, activity factor 2.3 -light activity (Whalan et al., 1997).

$$\text{Children: } U_{inh} = \frac{1 \times 21 \times 9.3 \times 22 / 24}{8} = 22.4 \text{ μg/kg bw/day}$$

BW = 8 kg, Inhalation rate = 9.3 m^3/day (children 0.5-3 year, respiratory volume while at rest 168 l/hour, activity factor 2.3 -moderate activity,Whalan et al., 1997).

Table 4.6 Calculated human exposure to DEHP from indoor air to adults and children

	Concentration in air (µg/m³)	U_{inh} Daily exposure per kg bw (µg/kg bw/day)
Indoor, PVC-paved room, adult	21	4.4
- " -, children	21	22.4

However, it is recognised that the calculated air concentrations are truly worst case estimates, and that if the risk characterisation would show that refinement of the exposure estimates is needed, there would be a need for reliable monitoring data.

Dermal exposure to DEHP caused by dermal contact with building materials such as flooring can be a significant route for children.

4.1.1.3.5 Gloves (clothing, gloves and footwear)

Much of the articles in this product group used in Europe are made in and imported from Asia. The knowledge of the used plasticisers and the release is limited. DEHP is used in a variety of products which contact human skin, these products include articles made of fabric coated by flexible PVC containing DEHP - clothing e.g. rain wear, plastic gloves and footwear e.g. high boots. The quantity of the human exposure to DEHP is a function of the area of skin in contact with the product, the duration of the contact, the surface availability of DEHP from the product and the percutaneous absorption of DEHP through the skin.

4.1.1.3.6 Dermal exposure

The amount of DEHP that a person is exposed to is a function of the area of skin in contact with the product, the duration of the contact, the surface availability of DEHP from the product and the penetration of DEHP through the skin.

The maximum internal exposure (U_{max}) by dermal contact to gloves is calculated using a dermal absorption rate (J) for contact with articles containing DEHP (Deisinger et al, 1998). See Model (I);

Model (I)

$$U_{\max} = \frac{J \times S_{derm} \times t_{\exp}}{\mathrm{BW}} = \frac{0.24 \times 840 \times 2}{60} = 6.7 \text{ µg/kg bw/day}$$

Alternatively, the calculation can account for the migration of DEHP from products (Hanson, 1983) and then the percentage absorption through the skin (5%) which has already been corrected for interspecies differences (see Model (II) below).

Data on the release of DEHP from products in contact with skin is limited.

Experiments designed to model the dermal exposure from contact with products containing DEHP have been performed. A flat vinyl product containing DEHP was scrubbed with a cotton cloth attached to a wood block. Experiments were conducted where the cotton was impregnated with lanolin to represent the oils present on human skin. The DEHP release value from the

product analysed is; R = 0.11 μg/cm^2/min (6.6. μg/cm^2/hour) (CMA, 1984). From this data the exposurer can be calculated with model (II), Hanson (1983).

Model (II)

$$C_{derm} = R \times t = 6.6 \times 2 = 13.2 \mu g / cm^2$$

$$U_{derm} = \frac{B_{derm} \times C_{\text{derm}} \times S_{\text{derm}}}{\text{BW}} = \frac{0.05 \times 13.2 \times 840}{60} = 9.3 \text{ μg/kgBW/day}$$

For both approaches it is assumed that the the skin contact area S_{derm} = 840 cm^2, contact duration; t = 2 hours/day, and persons body weight BW = 60 kg.

Comparison of the results derived from these two approaches are in close agreement. The value selected for the risk caracterisation without correction for interspecies differences is that based on the percutaneous adsorption rate derived from Deisinger et al. (1998) i.e model (I).

4.1.1.3.7 Car interior

Exposure to DEHP from car interiors occurs for both professional drivers and consumers.

The main route of exposure to DEHP in car interiors is assumed to be by inhalation, but dermal absorption may occur because of dermal contact with components made of plasticised PVC e.g. seats made of artificial leather.

The release of DEHP from details in the car interior is dependent on the temperature. Higher temperature results in a higher release.

If a car, with car seats made of DEHP-plasticised PVC in which doors and windows are closed is exposed to sunlight on a hot summers day, the release of DEHP may be significant. At higher temperatures DEHP will be expected to be in the gas/vapour form. At lower temperatures, the released DEHP will condensate either to form a mist or on surfaces. This surfaces can be either dust particles or components in the car interior.

The amount of DEHP used in a car interior may differ considerably depending on the kind of materials used e.g. for the car seats. Artificial leather made of PVC contains plasticiser which might be DEHP.

For the provided measured data of DEHP in car interiors, see below, no information on the materials used in the cars are submitted. Therefore it is not possible to establish if any of the measured values represents reasonable worst case.

Concentrations of DEHP in car interiors was reported in 1998 (Emissionen im Kraftfahrzeuginnenraum, 1998). The concentration of DEHP in six different cars was measured at room temperature. DEHP was detectable in two of the 6 cars. The concentration in a new car was 9.6 μg/m^3. After 20 days 5.2 μg/m^3 and after 40 days 1.8 μg/m^3. No information on interior materials used in the cars are provided. Therefore it is not possible to establish if the concentrations represent a reasonable worst case.

Measured values of the concentration of DEHP in car interiors from 1973 (Mieure, 1973), 1977 (McEwen, 1977 cited in General Motors, 1982b) and 1987 (Wams, 1987) have been submitted. The number of samples in the studies are not reported. In the Mieure study concentrations of

< 0.1 µg/m^3 at 25°C and 300 µg/m^3 at 60°C is measured. In the McEwen study concentrations from 0.2 to 1.2 µg/m^3 were identified in the interior atmosphere of a 1975 Opel. In the Wams study a concentration of 1,000 µg/m^3 is reported.

A concentration of DEHP in car interiors can be calculated using the saturated vapour pressure as a maximum gas concentration 5.3 µg/m^3 at 20°C. This may be an underestimation because release would be expected to be significant at higher temperatures and that DEHP condense forming an aerosol or condense on dust particles and in these forms expose the persons in tha cars.

Since a Norwegian study indicates that three times more DEHP is bound to particles in residence environments, we assume the same for car interiors. Then it can be inferred that 15.9 µg/m^3 (3 · 5.3) are adsorbed on to particles. The total air concentration may then be 21 µg/m^3.

Considering all the data available for car interiors, a reasonable worst case is estimated at 21 µg/m^3.

The daily exposure to DEHP in car interiors for adults and children is calculated, see **Table 4.7**. The body weight (BW) for an adult is 60 kg and 8 kg for a child. The inhalation rate (V_{inh}) for an adult is 20 m^3/day and 9.3 m^3/day for a child (Whalan et al., 1997).

$$U_{inh} = \frac{B_{inh} \times C_{inh} \times V_{inh} \times t / 24}{BW}$$

Values used for the calculation of inhalation exposure to airborne DEHP are as follow;

U is the uptake (mg/kg/day), B_{inh} the bioavailability for inhalation exposure (75% for adults and 100% for children), C_{inh} the air concentration (mg/m^3), V_{inh} the inhalation rate (m^3/day)), BW the body weight, and t_{exp} exposure duration (4 hours/day for adults and 2 hours/day for children)

$$\text{Adults: } U_{inh} = \frac{0.75 \times 21 \times 20 \times 4 / 24}{60} = 0.9 \text{ µg/kg bw/day}$$

BW = 60 kg, Inhalation rate = 20 m^3/day (adults 30-<60 year, respiratory volume while at rest 372 l/hour, activity factor 2.3 -light activity, (Whalan et al., 1997).

$$\text{Children: } U_{inh} = \frac{1 \times 21 \times 9.3 \times 2 / 24}{8} = 2 \text{ µg/kg bw/day}$$

BW = 8 kg, Inhalation rate = 9.3 m^3/day (children 0.5-3 year, respiratory volume while at rest 168 l/hour, activity factor 2.3 -moderate activity,Whalan et al., 1997).

Table 4.7 Calculated human exposure to DEHP from car interior to adults and children

	Concentration in air (µg/m³)	**U_{inh} Daily exposure per kg bw(µg/kg bw/day**
Car interior, adult	21	0.9
- " -, children	21	2

The main route of exposure to DEHP in car interiors is assumed to be inhalation, but dermal contact and absorption may also occur, eg in dermal contact with seats of plasticsed PVC.

Dermal exposure can be assumed to be more significant for children than for adults.

4.1.1.3.8 Exposure via medical treatment

There are many different types of PVC-based medical devices, including extracorporal blood circuits, e.g. for haemodialysis, plasma and plateletpheresis and blood oxygenation, infusion sets, catheters and endotractheal tube. DEHP is the principal plasticiser used for the manufacture of flexible PVC-containers for these applications. Over 95% of the total medical grade plasticised PVC is consumed in the manufacture of containers, flexible tubing and medical gloves (ECPI, 1994). Other materials used in medicine that contain DEHP include implantation materials e.g. artificial heart valves, gloves, catheters, syringes, several solutions and materials in ophthalmology and dentistry (Huber et al., 1996).

Humans may be exposed to DEHP through tubing and fluid storage bags used in medical practice. When receiving biological fluids i.e. nutrients, whole blood or blood components that have been stored in PVC containers, patients may be exposed to plasticisers via the intravenous route. PVC tubing used either for the administration of fluids or for dialysis may also contribute to the exposure to plasticiser (ECPI, 1994).

Catheters made of plasticised PVC used for feeding of infants, via the oesophagus, end in the gastric juice. This may result in significant release of DEHP in the stomach during the feeding period. No data on the magnitude of this release are available.

When lipid-containing fluids are stored in plasticised PVC-containers or conveyed through PVC-tubing the plasticiser will migrate from the PVC into the fluid. The rate of migration is dependent on the lipophilicity of the surfaces of the container and of the material stored, duration of storage, storage temperature, the extent to which the container is filled and the type of plasticiser used. **Table 4.8** shows the contamination of materials stored in PVC bags.

Two types of studies have been conducted in order to quantify human exposure to DEHP from medical devices. The first type measures the amount of DEHP that leaches from common medical devices, such as blood bags, into the physiologic medium that each device contains, such as blood. The second type measures the amount of DEHP or metabolites found in the blood, urine, or tissues of patients treated with medical devices containing DEHP.

Total DEHP exposure measured or estimated in this study varies significantly, although the exact reasons for the variability are unclear. The variability may be due to differences in study design and conditions, DEHP content in devices, and storage time.

Due to the wide spread use of articles for medical treatment and the assumed variations in treatment procedures, it is difficult to make a general assessment of the exposure. In addition, the populations exposed are inhomogeneous. Some of the persons in these populations are more vulnerable e.g. patients with impaired kidney function and patients with leukaemia. Premature infants receiving medical care may be exposed to DEHP during critical periods of development.

Table 4.8 Container-derived accumulation of DEHP in blood, blood products and stored fluids (Huber et al, 1996)

Material	Duration of storage	Temperature °C	DEHP (mg/l)	DEHP increase (mg/l/day)	Reference
Whole blood	< 3 w		24-110	0.8 - 3.9	Jaeger et al. (1970) Jaeger et al. (1972) Piechocki et al. (1973) Rock et al., (1978) Pik et al. (1979) NTP (1982) Ganning et al. (1984) Griffiths et al. (1978;1988)
Red cell concentrate	< 3 w		4-123		Plonait et al. (1993)
Red cell concentrate	5 w		174		Rock et al. (1987)
Platelet concentrate	2-5 d		180-650	100 - 200	Rock et al. (1978) Rubin et al. (1976) Labow et al. (1986) Shimizu
Plasma	1 w	4	< 110		Vessman et al. (1974)
Plasma	3 w	4	100-275		Vessman et al. (1974)
Plasma	10 w	4	< 890		Vessman et al. (1974)
Platelet- rich plasma	3 d	22	181		Rock et al. (1978)
Platelet-poor plasma	3 d	22	285		Rock et al. (1978)
Platelet-poor plasma	1 - 2 w	20	< 500		Dine et al. (1991)
Leucocyte-poor plasma	2 d		25-32	6.3 - 7.9	Piechocki et al. (1973)
Fluids for peritoneal dialysis	< 12 m	25	< 0.014		Nässberger et al. (1987)
Polysorbate 80 (surfactant)	1 d	24	36 - 237		Pearson et al. (1993)

Table 4.9 shows the amounts of DEHP released from different medical devices under patient-free conditions exposed in units of dialysis, transfusion and extracorporal oxygenation, without the patient being present.

Table 4.9 Extraction of DEHP or MEHP from units of dialysis, transfusion and extracorporal oxygenation (Huber et al, 1996)

Reference	Measure	Unit	
Dialysis	1.5 - 6	mg/h	Nässberger et al., 1987 Easterling,et al., 1974 Lewis,et al.,1978 Gerstoft et al.,1980 Bommer et al., 1981.
Dialysis	0.5	mg/l b.c.* after 5 h	Ono et al.,1975
Dialysis	1	mg/l b.c.* after 5 h	Ono et al.,1975
Blood transfusion	13 - 72	Mg extracted/l of blood given	Sjöberg,et al, 1985a
Blood transfusion	0.6 – 5 (MEHP)	Mg extracted/l of blood given	Sjöberg,et al, 1985a
Extracorporal oxygenation	3.5 – 4	mg/l extracted/h	Schneider et al., 1989

*b.c. Blood concentration

Table 4.10 summarises the long- and short-term medical procedures that implicate the most abundant exposure of patients to DEHP. The highest exposure to patients appears to originate from heamodialysis although some other treatments e.g. extracorporal oxygenation and blood transfusion, may temporarily lead to even higher exposure (Huber et al., 1996). However, heamodialysis is a special case, which is used for many months or even years usually for the treatment of kidney failure. It cannot be ruled out in patients with impaired kidney function that excretion of DEHP/metabolites in the urine may be impaired resulting in a longer retention and subsequent internal exposure.

Exposure may also occur to the hydrolysis product of DEHP, mono(2-ethylhexyl)phthalate (MEHP). Rock et al. (1978) showed that MEHP is formed from DEHP in the plasma during storage. Hence from plasma-exposure to DEHP + MEHP can occur simultaniously.

Table 4.10 Exposure of patients to DEHP resulting from medical treatments (Huber et al.,1996, ECPI, 1994, Doull et al.,1999)

Treatment	Short- long term exposure	Total exposure	Unit	Exposure (µg/kg bw/unit)	Average daily exposure (µg/kg bw/day)	Reference
Intravenous fluids (e.g. saline solutions, glucose solutions, nutrients)	Short	<1 mg/l				(Arbin et al., 1983)
Plasma	Short	7-120 mg/l				(Sasakawa et al., 19871978) (Rock et al., 1978) (Peck et al., 1979)
Whole blood	Short	20-70 mg/l				(Miripol et al., 1977)
Platelet and whole blood transfusion in infants	Long		year	2,100-27,500	6-75	(Jacobson et al., 1977)

Table 4.10 continued overleaf

Table 4.10 continued Exposure of patients to DEHP resulting from medical treatments (Huber et al.,1996, ECPI, 1994, Doull et al.,1999)

Treatment	Short- long term exposure	Total exposure	Unit	Exposure (µg/kg bw/unit)	Average daily exposure (µg/kg bw/day)	Reference
Transfusion of platelets					36 LADD[1)] 1	Doull et al., 1999
Neonatal transfusion					1,700	Hileman, 2000
Haemodialysis	Long	0,5-360 mg	session	10-7,200	4-3100	(Nässberger et al., 1987) (Lewis et al., 1978) (Ono et al., 1975) (Shneider et al., 1989) (Gibson et al., 1976) (Bommer et al., 1985) (Pollack et al., 1985) (Flaminio et al.,1988) (Ganning et al., 1984)
					457 LADD[1)] 99	Doull J et al., 1999
Peritoneal dialysis	Long	40 mg	year	800	2	(Nässberger et al., 1987)
Clotting factors in hemophiliacs	Long	2 mg	day	30	30	(CPSC, 1985 in Huber et al., 1986)
Blood transfusion in adults	Short	14-600 mg	treatment period	200-8,500		(Jaeger et al., 1972) (Sjöberg et al.,1985a) (CPSC, 1985 in Huber et al., 1986)
Blood transfusion in new-borns	Short		treatment period	500-4,200		(Sjöberg et al.,1985a) (Shneider et al., 1989) (Sjöberg et al., 1985b)
Blood transfusion in new-borns	Short		treatment period	50-700 (MEHP)		(Sjöberg et al.,1985a) (Sjöberg et al., 1985b)
Blood transfusion in new-borns	Short		treatment period	1,200-22,600		(Plonait et al., 1993)
Platelet concentrates in adults	Short	26-175 mg	treatment period	400-2,500		(Rubin et al., 1976) (Peck et al., 1978)
Platelet concentrates in new-borns	Short		treatment period	1,900		(Shneider et al., 1989)
Extracorporal oxogenation in infants	Short		treatment period	42,000-140,000		(Shneider et al., 1989)
Cardiopulmonary bypass	Short	2,3-168 mg	treatment day	30-2,400		(Jaeger et al., 1972) (Barry et al., 1989)

Table 4.10 continued overleaf

Table 4.10 continued Exposure of patients to DEHP resulting from medical treatments (Huber et al.,1996, ECPI, 1994, Doull et al.,1999)

Treatment	Short- long term exposure	Total exposure	Unit	Exposure (µg/kg bw/unit)	Average daily exposure (µg/kg bw/day)	Reference
Cardiopulmonary bypass	Short	0,25-19 mg	treatment day	4-300 (MEHP)		(Jaeger et al., 1972) (Barry et al., 1989)
Artificial ventilation in preterm infants	Short	0,001-4,2 mg	hour			(Roth et al., 1988)
Autopheresis -short term donor - long tem donor					 8.1 (LADD 1) 0.02 8.1 (LADD 1) 0.58	Doull J et al., 1999

1) LADD = lifetime average daily dose. Transfusion 2 years/lifetime, haemodialysis 15 years/lifetime

Summary/statement of human exposure to DEHP from medical products

Due to the intravenous exposure to DEHP during medical treatment, the uptake is 100%.

Table 4.11 shows the data for exposure to DEHP from medical products used in the risk characterisation.

Table 4.11 Exposure data for DEHP from medical products used for risk characterisation

Scenario	Average daily exposure (µg/kg BW/day)
1. Long-term haemodialysis (adults)	3,100
2. Long-term blood transfusion (adults) Haemophiliacs	30
3. Long term blood transfusion (children	75
4. Transfusions (neonates)	1,700
Lifetime average daily dose (LADD) Infusion of platelets Haemodialysis Autopheresis	 0.001 0.099 0.00058

4.1.1.4 Humans exposed via the environment

Food and drink may be contaminated both via the environmental and via non-environmental sources such as food contact materials. Because the media (i.e. food) and population are the same, for convenience, these results are presented in this section. The sources of human exposure to DEHP handled in this Section are food, water and air.

DEHP has been found in foodstuffs such as meat, fish and milk. Exposure of humans to DEHP through intake of food and drinking water may be due to general environmental contamination of the fresh food or contamination of food through the use of DEHP-containg materials in the handling and processing of the foood (e.g. in the food processing industry and from food packaging materials containing DEHP). Thus, the source of DEHP in food is not often known,

although there are cases where the use of PVC-tubings and gloves have been the major sources. Exposure of humans via inhalation of air may be caused by emissions of DEHP to the environment from different life-cycle steps, see Section. 2.1.

Residues present in breast milk originate from exposure of the mother at anytime up until breast feeding. Exposure of the mother potentially occurs from all DEHP sources.

No information on the natural occurrence of DEHP in foodstuffs is available.

DEHP may be released to the environment through waste water and air effluents at the sites where it is produced, transported, formulated, processed, used and after end-use of articles containing it. Indirect exposure of humans to DEHP via the environment may occur by intake of food, drinking water, and inhalation of air. Those indirect exposure routes via the environment are taken into account in Section 3.1.1.

The human intake from indirect exposure via food, water and air, in local and regional scenarios calculated with the EUSES-model are presented in the table below (**Table 4.12**).

There are recent biomonitoring data on the excretion of DEHP-metabolites in urine of different populations (Brock et al., 2002, Barr et al., 2003, US CDC 2003, Koch et al., 2003a,b,c, Koch et al., 2004). Based on the measured data and the knowledge of the fraction of DEHP that is excreted in urine, the total daily intake of DEHP (assumingly via the foood) can be calculated. By this approach, an estimate of the total exposure is obtained, including the contribution from handling and processing the food. Theoretically, these measured data should give more reliable intake values than the EUSES-model (but also higher values as 'industrial' contamination of the food also would be covered).

Several studies show that DEHP widely occurs in dairy products. One important source identified was DEHP-plasticised tubing in the milk transferring systems (Wildbrett et al., 1977, Castle et al., 1990, OAEI, 1996). However, one study indicated that DEHP occurs in a country were DEHP is no longer used as a plasticiser in transfer tubing (Sharman et al 1994). The author, therefore, suggests that the presence of DEHP in milk originates from an environmental source.

Monitoring studies show that DEHP was found in almost all dairy products (OAEI, 1996). The concentrations were between 1 and 7 μg/g fat. The average (excluding butter) was calculated to be 1.6 μg/g fat (=1.6 mg/kg fat). DEHP was found in butter with a range of 2.3 μg/g to 11.9 μg/g. A "background" level of 3-7 μg/g was analysed, when DEHP was not used in the wrapper (9.4 μg/g with the wrapper). DEHP was found in margarine with a range of 0.7 μg/g to 11.3 μg/g. The author stated that the "Background levels of DEHP (concentration measured in the food in the absence of DEHP in the packaging) were found to be greater in butter than in margarine, suggesting possible environmental contamination".

The concentration in fish has been reported to be up to 2.3-2.6 mg/kg (Pfannhauser et al 1997).

4.1.1.4.1 Biomonitoring of DEHP-metabolites in urine as a measure of the DEHP-intake

Measurements of urinary concentrations of DEHP-metabolites:

Urinary levels of a number of phthalates in the US population were reported by the US Centers for Disease Control and Prevention (US CDC, 2003). Samples from 2,541 individuals (from age 6 and older) in a US reference population were analysed for the concentration of MEHP, which

was reported both in ng MEHP/ml (=µg/l) urine and as µg MEHP/g creatinine. The geometric mean of the whole population was 3.4 µg/l (3.1 µg/g creatinine), and the 95th percentile 23.8 µg/l (18.5 µg/g creatinine). There were no differences between genders or ethnic groups. However, when compared with age of the persons (6-11, 12-19, and ≥ 20 years of age), the concentration was clearly highest in the children (e.g. 5.12 in children versus 3.21 µg/l in ≥ 20 years of age). The mean values were 60 or 71% higher (expressed as µg/l or µg/g creatinine, respectively) in the children (n = 328) than in the adults (n = 1,461), and the 95th percentile 54 or 139% higher in the children than in the adults.

In another paper from the US, CDC (Barr et al., 2003) have also utilised the urinary excretion of the secondary metabolites 5OH-MEHP and 5oxo-MEHP, in addition to MEHP, to assess exposure to DEHP in 62 people, including both children and adults. The population is not described in any more detail. The medians (and ranges) of concentrations were 35.9 µg/l (2.7-2,417 µg/l) for 5OH-MEHP, 28.3 µg/l (4.2-1,860 µg/l) for 5oxo-MEHP, and 4.5 µg/l (1.4-537 µg/l) for MEHP. Based on median concentrations, MEHP constituted 6.6% of the sum of the three metabolites analysed. The data are not presented adjusted for creatinine levels.

Brock et el (2002) reported on urinary levels of four phthalates (including MEHP) in Californian (US) children of age 11.8-16.5 months. Urine was collected from 19 children. The samples were treated with glucuronidase, and the free phthalate monoesters were analysed by LC-MS. Urinary creatinine levels were also determined and reported, but the phthalate monoester concentrations were only reported as µg/l urine. The mean concentration (± S.D.) of MEHP was 4.6 ± 6.4 µg/l, which is close to the data reported by US CDC (2003). The authors suggest that considering the lower urine volume and body weight of infants, the data indicate that the daily intake (per kg body weight) of DEHP in these infants may be at least twice higher than in adults. In some of the children, samples obtained at different days were analysed, showing up to 4-fold differences between the occasions.

The urinary concentrations of 5OH-MEHP, 5oxo-MEHP and MEHP were measured in 85 non-occupationally exposed Germans (7-64 years of age, median 33) (Koch et al., 2003c). The samples were analysed by LC/LC-MS/MS as described in a separate publication (Koch et al., 2003b). The medians (and ranges) of concentrations were 46.8 µg/l (0.5-818 µg/l) for 5OH-MEHP, 36.5 µg/l (0.5-544 µg/l) for 5oxo-MEHP, and 10.3 µg/l (< 0.5-177 µg/l) for MEHP. The corresponding creatinine-adjusted concentrations were 40.2 µg/g (6.9-449 µg/g) for 5OH-MEHP, 30.4 µg/g (6.4-262 µg/g) for 5oxo-MEHP, and 9.2 µg/g (< limit of quantification-123 µg/g) for MEHP. Based on median concentrations, MEHP constituted 11% of the sum of the three metabolites analysed. Females and males showed similar excretion characteristics for all DEHP metabolites. No significant correlation between DEHP metabolites and eating or drinking habits, body care habits, lifestyle or medication and medical history was found. Based on these data, Koch et al. (2003a) have estimated the DEHP-intake in this group (see below).

The internal exposure of nursery school children (aged 2-6 years, n = 36) to DEHP has recently been investigated by Koch et al. (2004) and compared to that of their parents and teachers (n = 19). MEHP, 5OH-MEHP and 5oxo-MEHP were determined in first morning urine. Creatinine adjusted total DEHP metabolites in urine were significantly higher in children than in adults (median values: 98.8 versus 50.9 µg/g creatinine; $p < 0.0001$). This also applied to the concentrations of the two secondary metabolites 5OH-MEHP (55.8 versus 28.1 µg/g creatinine; $p < 0.0001$) and 5oxo-MEHP (38.3 versus 17.2 µg/g creatinine; $p < 0.0001$). However, creatinine corrected concentrations for MEHP in children and adults were very similar (8.7 versus 8.6 µg/g creatinine; $p = 0.908$). Based on the sum of the three determined metabolites it was estimated that the DEHP dose (in µg/kg body-weight) taken up by the children was about twice as high as

the dose taken up by the adults. The study further demonstrates that calculating DEHP intake from the concentration of metabolites in urine may give erroneous results if MEHP is the only metabolite that is considered for the calculation. The mean relative ratio of MEHP to 5OH-MEHP+5oxo-MEHP in urine samples from the adult group was 1 to 5.5 while it was 1 to 12 in the children. This might indicate an enhanced oxidative metabolism in children. The biological significance of this is not clear as the toxicity of the various oxidative metabolites of DEHP has not been studied in any detail.

A higher exposure of children than of adults is also indicated by Barr et al. (2003), Brock et al. (2002) and US CDC (2003).

Based on the most recent studies (Barr et al., 2003 and Koch et al., 2003a,b,c,d), it appears that calculations of DEHP-exposure based on the excretion of metabolites gives more reliable results when several metabolites are measured (and not only MEHP). The reasons are that a larger share of the metabolites is measured and that the potential contamination of the samples with MEHP (e.g. artificially formed from DEHP during the enzymatic hydrolysis) can be overcome. The results by Koch et al. (2003a,b,c,d and 2004) and Barr et al. (2002) would thus be quantitatively more reliable than those of US CDC (2003), although the larger study group in the latter study is a clear advantage. It is also noted that the results from the studies on German and US populations show that the median concentration of DEHP metabolites in urine is higher in the former, thus indicating differences in DEHP exposure between the EU and the US, and advocating the use of EU-specific exposure data in the risk characterisation. Overall, we conclude that the German data by Koch et al (2003a,b,c) should be used for the risk characterisation.

Conversion factors for calculation of DEHP-intake based on urinary concentrations of DEHP-metabolites

To enable calculation of a DEHP-intake from data on the urinary concentrations of DEHP-metabolites, there is need for information from humans on what fraction of the given DEHP that is excreted as the different DEHP-metabolites in urine i.e. the conversion factors for the different metabolites. There are three studies that can be used for deciding the conversion factors (Schmid and Schlatter 1985, Anderson et al., 2001 and Koch et al., 2003d). These studies are described in Section 4.1.2.1.

There are shortcomings with all these studies, and there will be uncertainties with any choice of conversion factor. From the descriptions of the studies, it is apparent that the Anderson study (Anderson et al. 2001) is the one with the biggest shortcomings, as it studies a mixture of DEHP and DIOP, and as the secondary metabolites is not considered. In the opinion of the rapporteur, the DEHP-intake of people with a very fast further metabolism of MEHP is systematically underestimated if only MEHP is measured, as a small shift of the equilibrium between MEHP and it's two metabolites towards the metabolites will lead to a 10-fold bigger reduction of MEHP-concentration (the total concentration of the metabolites is approximately 10-fold higher than that of MEHP). It has been argued that there is greater person to person variability when secondary metabolites are measured. This seems to be the case in one of the studies (Barr et al., 2003) but not in the Koch et al. (2003a) study. Whether the variability is indeed bigger remains to be seen, but as the measurement of all three metabolites gives a more accurate and true picture of the human exposure, the rapporteur is of the opinion that the scientifically most sound approach to calculate DEHP-intake is to base it on measurements of all these three metabolites. The Schmid and Schlatter study (1985) involves all three major metabolites, but the urinary excretion is surprisingly low, and it may therefore lead to somewhat too low excretion factors. The study by Koch et al. (2003d) only involves one individual, but the use of the best available analytical techniques and deuterium-labelled DEHP lead us to propose to use the conversion

factors found in this study. Still, we note that the relative excretion of the three metabolites in this individual is slightly different from the ratios observed in different groups that have been studied (Koch et al., 2003a and Barr et al., 2003). The conversion factors proposed to be used are 7.3% for MEHP, 14.9% for 5oxo-MEHP, and 24.7% for 5OH-MEHP.

Calculation of DEHP-intake based on urinary concentrations of DEHP-metabolites

Koch et al (2003a) estimated the intake of DEHP in 85 non-occupationally exposed Germans, by measuring the concentration of 5OH-MEHP, 5oxo-MEHP, and MEHP in enzymatically hydrolysed urine samples. The study subjects were institute staff, their families and friends and students living in Erlangen and the vicinity. By measuring additional, secondary metabolites of DEHP and not only MEHP, more reliable data is achieved in this study than in other studies. The samples were analysed by LC/LC-MS/MS (Koch et al., 2003b) and the concentrations (reported separately in Koch et al 2003c) were transformed into intake levels of DEHP by using the fractional urinary excretion factors 0.074 for 5OH-MEHP, 0.055 for 5oxo-MEHP, and 0.024 for MEHP. The factors were derived from a study on the excretion of these metabolites after oral administration of DEHP to two human volunteers (Schmid and Schlatter, 1985), and the assumption that 25% of the daily dose of DEHP is excreted as metabolites in urine. The estimated median intake level was 13.8 µg DEHP/kg body weight/day, and the 95th percentile 52.1 µg/kg/day, when the calculation was based on the secondary metabolites. Males appeared to have higher levels (median 35% higher, 95th percentile 237% higher) than females but there were no correlations between levels and lifestyle habits. The study seems reliable, and the data for 3 out of 5 different phthalates are relatively close to previously published US data (US CDC, 2003). Higher intake levels of DEHP, and lower levels of DEHP, as calculated by Koch et al. (2003a), were found in the German study than in the material from the US study.

The rapporteur is of the opinion that the approach chosen by Koch et al. (2003a) to calculate DEHP intake from urinary concentrations of MEHP and MEHP-metabolites is sound, and that the data can be used if simultaneously considering the uncertainties in the obtained data. Others have advocated the use of a conversion factor of 13% (Anderson et al., 2001), which the rapporteur would not support for reasons which have been described. However, a new useful study, although only based on one individual, has been published, indicating a conversion factor of 7.3% for MEHP, and 24.7% for 5OH-MEHP, 14.9% for 5oxo-MEHP (Koch et al, 2003d). This new study, based on the most relevant techniques, would indicate that the conversion factor suggested by the Schmid and Schlatter study (1985) may be somewhat too low. Recalculation of the DEHP-intake in the Koch study (2003a) based on the new conversion factors and the secondary metabolites of DEHP results in a DEHP-intake threefold lower than originally reported i.e. a 95th percentile value of approximately 17 µg DEHP/kg body weight/day.

The rapporteur propose to use the 95th percentile intake value of 17 µg/kg/day based on German urinary concentrations of DEHP-metabolites (Koch et al. 2003a) and conversion factors obtained by Koch et al. (2003d). However, there is considerable uncertainty in this value as it is based on a conversion factor obtained from a single exposure of one individual, and as there are indications that children may be exposed to twice higher amounts of DEHP than adults. Still, a value of 17 µg/kg/day is brought forward to the risk characterisation as a measure of the regional exposure.

Breast milk

DEHP in human breast milk have been identified in two studies (Gruber, 1998 and Bruns-Weller, 2000). In the Gruber study, five samples showed concentrations of 71, 76, 79, 80

and 160 μg/kg milk and in the Bruns-Weller study five samples showed concentration of 20, 20, 110, 10 and 10 μg/kg milk. The median value for these 10 datapoints is approximately 75 μg/kg milk. It has been argued that the highest concentration (twice the median) is an outlier, but the variation can be compared with how the urinary concentrations of MEHP varies in different studies. In the US CDC study on 2,541 persons, the 95th percentile value is 7-fold higher than the mean (US CDC 2003), and in the study by Koch et al (2003) on 85 Germans, the 95th percentile value is 4-fold higher than the median. Thus, if assuming that there is a correlation between breast milk concentrations of DEHP and urinary concentrations of MEHP (which is likely), one may speculate that analyses of more milk samples is not likely to give a 95th percentile value less than the so far highest measured milk sample.

It is assumed that an infant breast feeds for 1 year, and that this year of life is subdivided into two periods (0 to 3 months and 3 to 12 months), reflecting the changing feeding demands of the infant. It is assumed that over the first 3 months the infant has an average weight of 6 kg, that the infant ingests 0.8 kg of milk per day, that 100% of the ingested DEHP is absorbed and that the breast milk has a fat content of 3.5%. From 3 to 12 months it is assumed that the infant has an average weight of 10 kg, that the infant ingests 0.5 kg of milk per day, that 100% of the ingested DEHP is absorbed and that the breast milk has a fat content of 3.5% (WHO 1998). It is also assumed that the content of DEHP remains constant during the breast-feeding period.

Using the following equation and the assumptions, as detailed above, the average daily uptake (U_{milk}) of the breast-feeding infant is estimated for both the 0-3 month and 3-12 month periods of infant life. The resultant uptakes are then summed to generate an average uptake for the infant in mg/kg/day.

$$U_{milk} = \frac{C_{milk} \times B_{ing} \times IR_{milk}}{BW_{infant}}$$

where:

$C_{milk,}$ represents the concentration of DEHP in mg per kg breast milk

B_{ing}, represents the bio-availability of the ingested DEHP (100/100 = 1)

IR_{milk}, represents the ingestion rate of milk by the infant (kg/day)

$BW_{infant,}$ represents the average infant body weight over the exposure period (kg)

DEHP in human breast milk have been identified in two German study (Gruber, 1998 and Bruns-Weller, 2000). The highest concentration measured (160 μg/kg) is used for calculation of reasonable worst case.

0-3 months: $U_{milk} = \frac{160 \times 1 \times 0.8}{6} =$ 21 μg/kg/day

3-12 months: $U_{milk} = \frac{160 \times 1 \times 0.5}{10} =$ 8 μg/kg/day

A study on the occurrence of DEHP in breast milk, blood, and urine of 42 Swedish women has recently been performed, and the processed but unpublished data has been made available to the Swedish rapporteur for the DEHP risk assessment (Hanberg et al, 2005). In that study, milk and the other samples were collected (in 2003) from women approximately two to three weeks after

they had given birth to their first child. The age of the women were 23-39, with a median age of 29. None of the women smoked during the pregnancy, but 5 before becoming pregnant. They all lived in the southernmost area of Sweden. The milk samples (50-100 ml) were collected, using DEHP-free pumps, into special glass bottles. Phosphoric acid was added and the samples frozen at –80^{o}C until analysed. Urine was also collected in glass bottles, but the urine was frozen at -80^{o}C without any additions. When leaving the samples, the women answered a detailed questionnaire, but the rapporteur has not seen any analyses of the answers. Milk and blood were analysed in 42 and 36 individuals, respectively, for DEHP and MEHP, whereas urine was analysed in 38 individuals for DEHP, MEHP, and two MEHP-metabolites. The blood data is not included in this evaluation. DEHP was analysed at IVL, Sweden. The milk samples were extracted with pentane:acetone (5:2), cleaned up with HPLC-GPC, the GPC-fractions were then extracted with hexane:MTBE, and finally analysed with GC/MS. Metabolites were analysed by the US CDC (the US Center for Disease Control), using their standard methodology (Barr et al, 2003).

Milk data

The mean (± S.D.) concentration of DEHP in milk was 17.1 ± 46.8 µg/liter milk. The MEHP concentration was 1.3 ± 1.3 µg/liter milk. The median values for DEHP and MEHP were 9.0 and 0.5 µg/liter, respectively, and the 95th percentile values 41.8 and 3.9 µg/liter, respectively. Considering that MEHP is probably the active testicular toxicant, it seems relevant to calculate the total concentration (sum) of DEHP (41.8 µg/l = 0.107 µM) and MEHP (3.9 µg/l = 0.014 µM), which becomes 0.121 µM, or if re-converted into DEHP (using the DEHP molecular weight) to facilitate the comparison, 47.3 µg/liter milk at the 95th percentile level (Hanberg et al, 2005).

In the two old studies (Gruber 1998, Bruns-Weller 2000), the concentration of DEHP in 10 women ranged from 10 to 160 µg/kg milk, with a median value of approximately 75 µg/liter. Assuming that one liter milk is equivalent to one kg milk, the current Swedish concentration of DEHP+MEHP is approximately 8 or 3.5-fold lower than the old data, depending on comparing median values or the current 95th percentile value with the old maximum value.

For a newborn (0-3 months of age) a milk concentration of 47 µg/liter results in a daily exposure to 6.2 µg/kg/day of DEHP and MEHP (see formula above), which is taken forward to the risk characterisation. At an age of 3-12 months, the corresponding intake becomes 2.4 µg/kg/day.

In order to assess how representative the Swedish milk data is for the EU exposure situation, the exposure of Swedish women to DEHP has to be compared with data from other countries within the EU. As the urinary excretion of DEHP-metabolites is believed to correspond to the exposure situation, the urinary DEHP excretion in the Swedish women is compared with recent data from a German population.

Urine data

In the urine from the Swedish women, no DEHP was found, which is in conformity with other studies. The mean (±S.D.) urinary concentrations of MEHP, 5OH-MEHP, and 5oxo-MEHP were 13.3 ± 10.2, 25.3 ± 26.5, and 18.6 ± 19.2 µg/liter urine, respectively. The median values were 9.0, 14.6, and 11.4 µg/liter urine, respectively, and the sum of the median values for the three metabolites 35 µg/l (because of rather similar molecular weights, the addition is based on µg even though not scientifically correct).

It can, thus, be concluded that the Swedish urinary concentrations of DEHP-metabolites (sum of median concentration of the three metabolites is 9.0 + 14.6 + 11.4= 35 µg/liter urine) are roughly

similar (within a factor of 3) to the concentrations in Germany (10.3 + 46.8 + 36.5= 93.6 μg/liter urine) (Koch et al 2003c). However, it is noteworthy that the ratios between the three metabolites differ somewhat. The reason for this is unknown. Assuming that the metabolism of DEHP does not differ too much between the general German population and the Swedish women (which have recently been pregnant and delivered a child), the urinary excretion indicates similar exposure-levels to DEHP. Consequently, the data on the occurrence of DEHP and MEHP in breast milk in Swedish mothers is most likely representative for the EU-situation.

A recent study reports on the occurrence of MEHP in breast milk of Danish women and in a few samples of consumer (cow) milk and infant formulas (Mortensen et al, 2005). The study focuses on the development and validation of a quantitative analytical method for measuring phthalate metabolites in human breast milk, but it also contains data from real samples. Reasons for only analyzing monoester metabolites are claimed to be that the monoesters are the active testicular toxicants and that by not looking for the parent phthalate one avoids the problems with contamination of the samples with the parent phthalate from the environment. Breast milk samples (n=36) were collected during 1997-2001, originally for other research purposes, 1-3 months after the women had given birth. No information on the mothers is given. In addition, seven samples of common consumer milk (1.5-3.5% fat content), and ten samples of infant formula products were collected, presumably recently although the year is not stated.

Breast milk samples were generally collected directly into a glass or porcelain cup, but sometimes using a breast pump. The samples were directly frozen at –20^{o}C and kept at -20^{o}C until analysed. No acid was added to the samples at the time of sampling, leading to some uncertainty as to the possible enzymatic hydrolysis of DEHP to MEHP after the sampling. The samples were analysed at the Copenhagen University Hospital in Denmark. The milk samples were extracted with ethyl acetate:cyclohexane (95:5), cleaned up twice with solid-phase extraction cartridges, dissolved in 10% acetonitrile, and analysed with LC-MS-MS. In total, six different phthalate monoesters were looked for; MEHP, monomethyl phthalate, monoethyl phthalate, mono-n-butyl phthalate, monobenzyl phthalate, and monoisononyl phthalate.

Breast milk data

The mean (±S.D.) concentration of MEHP in milk was 13 ± 11 μg/liter milk, the median 9.5 μg/liter (corresponding to a median concentration of 0.4 μg/g lipid), and the range 2.7-72 μg/liter. The 95th percentile value is not reported. The report says that there is no significant difference in MEHP concentration between samples collected with or without breast pump (but no data is given), indicating negligible contamination of the samples with MEHP from the plastics and, if contamination with DEHP had occurred, limited hydrolysis of DEHP to MEHP. Addition of phosphoric acid to the samples at the time of thawing for the analyses did not significantly affect the concentration of MEHP, not even when incubating the samples for up to 24 hours prior to the analyses. There is a possibility that some hydrolysis could have occurred after sampling , but prior to freezing, but the report states that there was only a "short time" (not defined) available for this potential hydrolysis, thus potentially only leading to minimal hydrolysis of DEHP to MEHP. The authors conclude that the MEHP concentrations found are not being overestimated by an artifactual hydrolysis of DEHP to MEHP after the sampling. The authors have been further consulted on this issue, and although they can not completely rule out some hydrolysis in the old samples before they were frozen, the authors claim to have done control experiments (not published) showing that no hydrolysis occurs in newly sampled breast milk not preserved with acid when keeping them at room temperature for up to one hour.

For the other phthalates measured, it can be noted that the median concentration of mono-n-butyl phthalate and monobenzyl phthalate in the breast milk was somewhat lower than that of MEHP (within a factor of 10).

Consumer milk and infant formula data

The MEHP concentrations are only reported as ranges, and the ranges are 5.6-9.1 μg/liter for consumer milk and 7.7-9.9 μg/liter for infant formula. The order of magnitude is, thus, similar to the one in the breast milk samples, but the variation is much smaller than in the breast milk samples. For the other phthalates measured, the concentration of mono-n-butyl phthalate was somewhat lower than that of MEHP (within a factor of 10), whereas no monobenzyl phthalate could be detected.

In the Gruber (1998) and Bruns-Weller (2000) studies, the concentration of DEHP in 10 women ranged from 10 to 160 μg/kg milk, with a median value of approximately 75 μg/liter (MEHP was not measured). In the recent Swedish study (2005), the median values for DEHP and MEHP were 9.0 and 0.5 μg/liter, respectively. The Danish median concentration of MEHP of 9.5 μg/liter is, thus, 19 times higher than the Swedish median concentration of MEHP. If comparing mean concentrations of MEHP in the Danish and Swedish groups, the concentration is 10 times higher in Denmark than in Sweden (13 versus 1.3 μg/liter). If comparing the upper range values, there is a 18-fold difference between the maximum Danish value and the 95th percentile Swedish value. Such a big difference in MEHP concentrations between two neighboring countries is unexpected and not easy to explain. Perhaps, it could be related to the fact that the Danish samples are a few years older than the Swedish. More likely, it could be caused by hydrolyses of DEHP to MEHP in the Danish samples. However, the US CDC (Calafat et al, 2004) has recently reported similar concentrations of MEHP in three American pooled breast milk samples (mean ± S.D. of 7.8 ± 6.8 μg MEHP/liter milk) as reported in the Danish samples. Calafat et al (2004) also reported on the presence of MEHP-metabolites in the breast milk, although at concentrations close to the limit of detection. However, it should be noted that the US samples were not preserved with acid immediately at the time of collection.

If assuming a 95th percentile concentration of 35 μg MEHP/liter for the Danish mothers (based on the range of 2.7-72 μg/liter, and calculated as mean + 2 SD), a newborn (0-3 months of age) would be exposed to 4.6 μg/kg/day of MEHP (see formula above), without considering the unknown contribution from the DEHP also expected to be present in the milk.

Measured data for food

DEHP concentrations in a variety of foods have been reported. The concentrations found are presented in Annex 2. In a recent review (Huber et al., 1996) exposure of man to DEHP from environmental sources has been reviewed. The article identifies different sources of exposure, the environmental concentrations, amount available for potential internal exposure (e.g. inhaled amount) and the internal exposure. The article also identifies highest exposures ("most relevant data") and worst case scenarios.

The concentration of DEHP in fish has been reported to be up to 2.3-2.6 mg/kg (OAEI, 1996 and Pfannhauser et al., 1997). In fresh water fish in Austria in 1997 a total of 180 fishes were collected at 58 locations (Pfannhauser et al. 1997). DEHP was the only plasticiser found in all of the 71 (39.4%) positive samples containing measurable amounts of phthalates. The highest level of DEHP found was 2.6 mg/kg (WWT) in carp. At five sites DEHP levels in a total of eight fish samples exceeded 1 mg/kg (WWT). The 90th percentile was approximately 0.5 mg/kg (WWT). In a survey in The Netherlands (RIC, 2000b) 25 fish samples from different locations were

analysed for phthalates. The DEHP concentrations ranged from < 0.001 mg/kg to 0.149 mg/kg (WWT) with a 90th percentile of 0.089 mg/kg. These values are lower than those from the Austrian study. However due to the fact that fewer samples were taken and that the samples were stored for two years prior to analysis these values are considered less reliable. In a complementary study (RIC, 2001d) three fish samples gave DEHP concentrations of approximately 0.3 mg/kg (wwt) in all three samples.

In a Danish study (Petersen, 2000), the plasticizers DEHP, di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), and di-2-(ethylhexyl)adipate (DEHA) were analysed in 29 total diet samples, 11 samples of baby food, and 11 samples of infant formulae. In all of the total diet samples, the presence of one or more of the plasticizers was demonstrated. DEHP was the plasticizer determined most frequently in total diet samples (11 above limit of determination out of 29). Maximum and minimum mean concentrations in the total diet samples were 0.11-0.18 mg DEHP/kg. The highest concentration of DEHP in a total diet sample was 0.49 mg DEHP/kg. Based on a total daily intake of 10 MJ (energy content in food) this sample gave an exposure of 1.1 mg/day. This is equivalent to an exposure of 16 µg/kgBW/day for a person of 70 kg BW.

Tsumura et al. (2001a) studied the concentration of DEHP in marketed prepacked lunches (n=16) and set lunches (n=10) in Japan, by extracting DEHP from the homogenised meals with acetonitrile, and analysing by GC/MS. The concentration of DEHP was 0.8-11.8 mg/kg in packed lunches and 0.012-0.30 mg/kg in set lunches. By following the concentration of DEHP in food during the processing, they showed that handling the food with disposable PVC gloves (containing DEHP) increased the concentrations of DEHP in the food. Experiments showed that the leakage of DEHP from the gloves to the foodstuff was pronounced, especially after ethanol sterilisation (spraying) of the gloves.

When duplicate diet samples from Japanese hospitals were analysed in 1999, the range of concentrations of DEHP was 10-4,400 µg/kg food (Tsumura et al 2001b). When similar hospital duplicate diets were analysed two years later, after the use of PVC gloves for food had been regulated (Tsumura et al 2003), the concentrations were 6-675 µg/kg food (range). The mean intake (estimated from all samples) were 519 µg DEHP/day/person before the regulation and 160 µg DEHP/day/person after the regulation. Assuming a body weight of 60 kg, it approximately corresponds to a mean daily exposure to 9 and 3 µg/kg/day, respectively, via the hospital diets. High concentrations of DEHP (mean 5,990 ng/g) were found in one brand of baby food (recommended for babies older than 7 months) (Tsumura et al 2001b). The consumption (by a 8 kg baby) of one package per day (80 g/day) would result in a mean exposure of 59.9 µg/kg/day. The source of contamination was identified as a PVC-tube used during production. Replacing the tube by one made of stainless steel effectively reduced the DEHP contamination (by more than 90%). The Japanese data show that high concentrations of DEHP in food can be obtained by contamination during handling and processing of the food. PVC gloves are likely used in the EU as well, although the extent to which they are used in food handling is not known.

Water

Due to the low water solubility of DEHP the exposure is very low, although individual instances of contamination may be as high as 170 µg/l. In contaminated groundwater in the Netherlands levels from 20 to 45 µg/l have been reported (WHO, 1992).

Modelled

The EUSES program includes a model on the concentration of a chemical in biota which have relevance for the food chain. Intake can be determined based on the information of the concentration in the food and the intake data such as in EUSES. The indirect exposure of humans to DEHP originates from several sources. The exposure assessment (EUSES) includes six pathways: drinking water, fish, crops, meat, milk and air. The daily dose for humans is calculated by means of the concentrations in these media and the daily intake values. The default consumption rates for each food product are given in **Table 4.12**. These values represent the highest country-average intake across all EU Member States for each food product. The children's intake is assumed from data on the food habits of the Swedish population in 1989 (Becker, 1989) and data on the daily energy intake for different ages (MAFF 1998d)

Table 4.12 Daily human intake (adults and children) of drinking water, different foodstuff and daily inhalation rate

Parameter	Value Adult	Value Child	Unit
Daily intake of drinking water	0.002	0.001	m^3/day
Daily intake of fish	0.115	0.084	kg_{wwt}/day
Daily intake of leaf crops (incl. fruit and cereals)	1.20	0.6	kg_{wwt}/day
Daily intake of root crops	0.384	0.192	kg_{wwt}/day
Daily intake of meat	0.301	0.229	kg_{wwt}/day
Daily intake of dairy products	1.333	1.68	kg_{wwt}/day
Daily inhalation rate	20	9.3	m^3/day
Body weight	70	8	kg

The human exposure to DEHP via the environmental routes has been estimated both regionally, and locally for all life-cycle stages, using EUSES 1.0.

The concentration of DEHP in milk used in this risk caracterisation, is 0.05 mg/kg based on Danish milk analysis (Petersen, 1991). This value is in good agreement with data from two farms in The Netherlands (ECPI, 2000) where concentrations in milk ranged from 0.009 to 0.07 mg/kg with a 90^{th} percentile of 0,05 mg/kg. In the further calculations with EUSES the measured concentration in milk (0.05 mg/kg) is used both for the regional and local scenarios. This value is used for the local scenarios because the contamination of milk in the local scenarios may not only originate from the local point source but also e.g. from fodder or from the processing of the milk in the dairy.

The total daily intake via air, drinking water and food at local scale and regional scale calculated by EUSES are summarised in **Table 4.13**, **Table 4.14** and **Table 4.15**.

Table 4.13 Total daily intake via air, drinking water and food at local scale

Life cycle stage or scenarios	Total daily intake. Child. (mg/kg/d)	Total daily intake. Adult (mg/kg/d)
Production		
Site 1	0.0194	0.00189
Site 2	0.0278	0.0032
Site 3	0.0186	0.00172
Site 4	0.0195	0.0019
Site 5	0.0384	0.006
Site 6	0.0522	0.008
Site 7	0.0189	0.0018
Site 8	0.022	0.0023
Site 9	0.0186	0.00175
Site 10	0.0184	0.00169
Site 11	0.0382	0.00483
Site 12	0.0407	0.00538
2 Processing of polymer products		
2a. Processing of polymer products; *calendering*	0.215	0.0452
2b. Processing of polymer products; extrusion compound	0.105	0.0201
2c. Processing of polymer products; extrusion products	0.105	0.0201
2d. Processing of polymer products; spread coating with air cleaning	0.118	0.0231
2d. Processing of polymer products; spread coating without air cleaning	0.312	0.0671
2e. Processing of polymer products; other plastisol with air cleaning	0.077	0.014
2e. Processing of polymer products; other plastisol without air cleaning	0.277	0.0593
3 Non-polymer processing/formulation		
3a. Non-polymer. Sealants formulation	0.292	0.0626
3b. Non-polymer. Sealants processing/application	0.0195	0.00194
4a. Non-polymer. Laquers and paints	0.244	0.0519
4b. Non-polymer. Laquers and paints processing/application	0.042	0.00659
5a. Non-polymer. Printing inks formulation	0.245	0.0522
6. Non-polymer. Ceramic formulation	0.032	0.00452
7a. Municipal STP	0.0831	0.0148
8a. Printing inks, paper recycling	0.0869	0.0161
8b. Waste. Car shredder	0.0188	0.00178
8c. Waste incineration	0.0206	0.00219

Table 4.14 Total daily intake via air, drinking water and food for children at sites (life cycle stages 2 and 3) for which site specific emission data are available

Life cycle stage and site	Total daily intake. Child. (mg/kg/d)
2a Calendering	
Site F3	0.0233
Site S4	0.034
SiteS6	0.031
2b Extrusion compound	
Site S5	0.0187
2c Extrusion product	
Site F2	0.0286
Site F7	0.0224
Site I9	0.0236
Site S11	0.0187
2d Spread coating	
Site F1	0.025
Site S8	0.0444
2? Polymer, not known	
Site ES12	0.0233
Site ES13	0.0205
3a Sealants/adhesives form	
Adhesives Site F10	0.0259

Table 4.15 Regional scale concentrations and total human intake as estimated by EUSES1.0

	Children	Adult
Intake (mg/kg/d)	0.0194	0.00193

The predominant contribution to total exposure for adults in the regional scenario is from fish (52%), dairy products (24%) and root crops (18%) and for children, fish (31%), dairy products (58%) and root crops (7%).

4.1.1.4.2 Summary/Conclusions

Exposure via out-door air

Inhalation of air out-doors may cause human exposure to DEHP, caused by the emissions from the industry handling DEHP and materials containing DEHP used in the society. Exposure to DEHP via inhalation of ambient, out-door air is generally considered a minor source. Due to the low vapour pressure the exposure is very low.

DEHP in the atmosphere can either be adsorbed to particular matter or be in the vapour phase. Giam et al 1980 found that more than 50% of the atmospheric DEHP is in vapour form.

The concentration and the human exposure to DEHP via air have been calculated with EUSES 1.0.

Exposure via food, water and air - Internal exposure

The exposure has been assessed by two different approaches; based on measured urinary excretion of DEHP-metabolites, and with the EUSES model.

Daily regional exposure to DEHP has been estimated based on measured urinary excretion of DEHP-metabolites in a German population (Koch et al., 2003a). The report calculates a 95th percentile intake value of 52.1 µg/kg/day based on excretion conversion factors of 5.5 and 7.4% for the two secondary metabolites of MEHP. When recalculated using the new conversion factor of Koch et al. (2003d), a 95th percentile intake value of 17 µg/kg/day is obtained. The calculated intake value of 17 µg/kg/day (based on MEHP-metabolites), which will be brought forward to the risk characterisation, can be compared with measured DEHP concentrations in food from Denmark and Japan, indicating intake levels up to 16 µg/kg/day and 59.9 µg/kg/day, respectively. Biomonitoring should theoretically include exposure through all different routes.

The EUSES-calculated exposure is based on both estimated (most foodstuff) and measured food (milk) concentrations, and the food consumption. Exposure is calculated based on daily intake of different foods, water and air. For adults a body weight of 70 kg and inhalation rate of 20 m^3/day is used. For children a body weight of 8 kg and inhalation rate of 9.3 m^3/day is used.

Infant formulae

A MAFF survey estimated the intakes of individual phthalates and total phthalates by infants from infant formulae (MAFF #83 1996b). A total of 59 individual samples of 15 different brands of infant formulae were analyzed. The level of DEHP, which was the more abundant phthalate found in samples, ranged from 0.33-0.81 mg/kg (dry powder) (casein dominant formulae), 0.38-0.98 mg/kg dry (whey-dominant) and 0.38-0.56 mg/kg dry (soy-based).

The more recent MAFF study estimated the intakes of individual phthalates and total phthalates by infants from infant formulae (MAFF #410 1998c, MAFF #168 1998a). A total of 39 individual samples of 14 different infant formulae products (powdered and ready-to-feed) were analysed. Levels of all phthalates measured are lower than those reported for the previous survey in 1996 (MAFF #83 1996b). But as in 1996, DEHP was the most abundant individual phthalate and the level ranged from 0.05-0.44 mg/kg (dry powder) (MAFF #410 1998c, MAFF #168 1998a). The concentration of phthalates in infant formulae has also been reported from one Danish study (Levnedsmiddelstyrelsen, 1996 and Petersen and Breindahl, 2000) and one German study (Gruber, 1998). In the Danish study the concentration of phthalates in 13 different products on the Danish market was analysed. The concentration of DEHP in infant formulae in the Danish study was 0.004-0.06 mg/kg wet weight. The highest estimated intake of DEHP calculated was 0.015 mg/kg BW/day. This exposure was calculated from the recommendations given on the package for the product (0.75 L/day) and a bodyweight of 3 kg. In the Gruber study (1998), 8 samples of infant formulae were analysed for DEHP. The concentration was between < 0.05-0.196 mg/kg dry weight (mean 0.13 mg/kg). If the daily intake of the product is assumed to be 81g (dry) and the body weight 2.5 kg, the internal exposure is calculated to be < 0.002-0.006 mg/kgBW/day.

The results of the study conducted by MAFF 1998, with support from the Danish and German studies, show a decrease in the concentration of DEHP in infant formulae in relation to the measured concentrations in the MAFF study from 1996. The maximum level of DEHP in samples analysed by MAFF in 1998 is 440 µg/kg dry powder (MAFF #168 1998a). This concentration is used as a reasonable worst case in the risk caracterisation. The internal exposure to DEHP present in infant formulae at six different ages is shown in **Table 4.16**. The daily intake of the infant formulae is based on the recommendations given on the package of the product and information on the weight of the formula per scoop (see MAFF 1998a,c, #168 and 410, MAFF 2000). The exposure of infants, 0-3 months old, is calculated from the data for the first four youngest age groups. The calculated exposure of babies around 3 kg (0.014 mg/kgBW/day) is similar to the exposure (0.015 mg/kgBW/day) derived for 3 kg babies in Denmark (Levnedsmiddelstyrelsen, 1996 and Petersen and Breindahl, 2000)

The exposures for the ages 0-3 months and 6+ months are used for calculations of MOS in the risk characterisation. Data for the actual product for which the calculations are made is not available for the ages 4-6 months.

Table 4.16 Infants exposure to DEHP by infant formulae at six ages

Age	Concentration DEHP (mg/kg dry weight)	Bodyweight (kg)	Total intake infant formulae -dry weight, (g)*	Exposure, mg/kgBW/day
0-1 week	0.44	2.5	81	0.014
2-4 weeks	0.44	3.5	108	0.014
1-2 months	0.44	4.5	135	0.013
2-3 months	0.44	5.5	135	0.011
3-4 months	0.44	6.5	158	0.011
4-6 months	0.44	No data	No data	-
6+months	0.44	8	144	0.008
#0-3 months	0.44	4.4	124	0.013

* The intake for the different ages are calculated based on recommended doses on the package for the product and information on the weight of formulae per scoop.

\# Calulated based on data for the first four youngest age groups. The bodyweight and the total intake are calculated as time weighted averages.

Summary

The external and internal exposure of DEHP for the chosen populations and the worst case scenarios for the risk characterisation are summarised in **Table 4.17**.

4.1.1.5 Multiple pathways

Human exposure to DEHP of different populations and sub-populations by multiple exposure routes is possible (multiple pathways).

The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP are presented in **Table 4.17**.

The exposure to DEHP can be by different routes - inhalation, dermal, oral and intravenous. In some cases the individual may be exposed by more than one route at the same time.

Some of these situations are identified:

- Occupational exposure (inhalation and dermal) when handling the pure substance during production and formulation.
- Consumers exposure when children play with toys containing DEHP (oral and dermal).

The extent of exposure by multiple pathways is calculated as the sum of the highest exposure from each route from all sources during a day. The total multiple routes exposure is regarded as a worst case less probable than the individual exposures by each route.

Table 4.17 The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m^3)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Occupational								
Production of DEHP	5	530	650	460				990
Industrial use of DEHP	10	1,060	420	300				1,360
Industrial end- use of products containing DEHP	10	1,060	1,300	928				1,988
Consumer								
- Adult								
”Indoor air” (building materials)	0.021	4.4						4.4
Gloves				6.7				6.7
Car interior	0.021	0.9						0.9
Multiple pathways of exposure		5.3		6.7				12
- Children								
”Indoor air” (building materials)	0.021	22.4						22.4
Toys and child-care articles				9	1.6	200		209
Car interior	0.021	2						2
Multiple pathways of exposure		24.4		9		200		233.4
Medical								
Haemodialysis (adults; long-term)							3,100	3,100
Blood transfusion (adults) (short-term)							8,500 μg/kg bw/unit	

Table 4.17 continued overleaf

Table 4.17 continued The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m^3)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Blood transfusion (new-borns) (short-term)							22,600 μg/kg bw/unit	
Extracorporal oxogenation in infants (short-term)							140,000 μg/kg bw/unit	
Peritoneal dialysis (long-term)							2	2
Clotting factors in haemophiliacs (adult; long-term)							30	30
Neonatal transfusion							1,700	1,700
Platelet and whole blood transfusion in infants (long-term)							6-75	6-75
Lifetime exposure (LADD) [1)]								
Infusion of platelets							1	1
Haemodialysis							99	99
Autopheresis							0.58	0.58
Human exposed via the environment[2]								
Adult. Total –food, water and air, local (e.g. exposure via STP, scenario 7a)-EUSES								14.8
Adult. Total –food water and air, regional-EUSES								1.93

Table 4.17 continued overleaf

Table 4.17 continued The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m^3)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Adult. Total –regional, based on biomonitoring[3]								17
Child. Total –food, water and air, local (e.g. exposure via STP, scenario 7a)-EUSES								83
Child. Total –food, water and air, regional-EUSES								19.4
Infants exposed via infant formulae and breast milk								
Infant formulae (0-3 months)						13		13
Infant formulae (6+ months)						8		8
Breast milk (0-3 months)						6.2		6.2
Breast milk (3-12 months)						2.4		2.4

1) LADD=lifetime average daily dose. Transfusion 2 years/lifetime, haemodialysis 15 years/lifetime.
2) The exposure indirectly via the environment goes via the oral route and inhalation (air). The oral exposure is the main exposure route (> 99% of the total exposure via the environment).
3) Based on biomonitoring (urinary excretion) of the general population. Includes exposure also from other sources than food and outdoor air.

4.1.1.6 Combined exposure

Combined exposure of different populations and sub-populations is also possible and may occur within the separate populations/sub-populations either at different times or together.

It is also recognised that sequential or separate exposure to other phthalates may also occur and the possibility that interactive effects may occur is considered an issue. Due to the wide use of DEHP in the society and the diffuse emissions from the products, humans may be exposed from many different sources. The total exposure (body burden) is the sum of all the specific exposures from all sources by all routes, however, all sources of human exposure to DEHP have not been quantified or identified. Therefore the total combined exposure is probably higher than the calculated sum of the specific exposures from the different sources.

The exposure values presented in this report are for the mean exposure. No information is available on peak exposures, frequency and duration, this makes it difficult to calculate a total combined exposure. Other exposure can occur due to misuse of products containing DEHP e.g. heating food in plastic containers in micro-wave ovens. No data on this exposure is available.

In this assessment four scenarios of combined exposure are chosen see **Table 4.18**.

Humans may also be exposed to other similar chemicals e.g. other phthalate esters, which can cause synergistic effects. One example is children playing with toys which can contain one or more phthalate esters as plasticisers. The wide use of these substances in the society makes multiple chemical exposure possible.

Table 4.18 Combined exposure

Population	Source	Adult. Occup.	Adult. Non-Occup.	Child	Babies/ infants
Occupational		X			
Consumer	Toys and children´s articles			X	X
	"Indoor air" (wall and floor covering)	X	X	X	X
	Gloves	X	X		
	Medical	(X)	(X)	(X)	(X)
Via the environment	Food, water and air	X	X	X	X
	Infant formulae				X
	Breast milk				X

X Indicates exposure
(X) Indicates possible additional exposure

4.1.1.7 Summary

Exposure of children is considered separate from other non-occupational populations.

In addition, internal exposure is determined when not directly available from information for each population/subpopulation. It is also recognised that all exposure groups may not have been identified since it has been impossible to obtain information on all potential exposure situations for DEHP within the European Union. Therefore, it is recognised that not all exposure scenarios may have been covered.

The populations, the routes of exposure, the kinds of exposure data and the reasons for selection of exposure data used for the calculation of MOS are summarised in **Table 4.19**.

Table 4.19 Summary of reasons for selection of exposure data used for the calculation of MOS

Population	Routes	Measured	Calculated	Modelled	Rationale
Occupational					
Production	i	X			Measured exposure values used because of limited number of well defined ”large” sites producing DEHP
	d			X	No measured data.
Industrial use/ Processing	i	X			
	d			X	No measured data.
Industrial end-use	i	X			Few data but because of the constant environment in a vehicle this was accepted to be constant. However, that data are old
	d			X	No measured data
Consumer					
Adult:					
PVC gloves	d		X		Worst-case, no other data
Indoor air	i		X		Realistic worst-case, no other data
Child:					
Toys etc.	o		X		Realistic-worst-case
	d		X		Realistic worst-case
Indoor air	i		X		Worst case, no other data
Medical products					
Adult:					
Dialysis	i.v.	X			Measured data used because well defined use with constant variables
blood trans. (ch)	i.v.	X			Measured data used because well defined use with constant variables
blood trans. (ac)	i.v	X			Measured data used because well defined use with constant variables
Child:					
extra corp. oxy	i.v.	X			Worst-case. Qualitative
Food, water, air					
Adult:					
all sources	o	X			Measured data + kinetic data
	o	X		X	Measured data + EUSES
	i	X		X	Measured data + EUSES

Table 4.19 continued overleaf

Table 4.19 continued Summary of reasons for selection of exposure data used for the calculation of MOS

Population	Routes	Measured	Calculated	Modelled	Rationale
Food, water, air					
Child:					
all sources	o	X		X	Measured data + EUSES
	i	X		X	Measured data + EUSES
infant formulae	o	X			Measured data.
Mothers milk	o	X			Measured data

i) Inhalation
o) Oral
d) Dermal
i.v.) Intra-veinous

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

Numerous studies on the toxicity of di(2-ethylhexyl) phthalate (DEHP) have been performed in experimental animals. A number of the available studies have been omitted from the risk assessment report because of their limited quality or relevance with respect to the risk assessment.
Very few studies have been performed according to Annex V. A number of studies are, however, comparable to guideline studies and several studies have been performed under GLP conditions; the information is included in the text.

When expressing results, the term "significant" is used only if the results are statistically significant at a p-level lower than 0.05.

The term "guideline studies" is used in the following text to donate studies conducted according to the guideline of Annex V to the Council Diretive 67/548/EEC, or comparable to guideline studies.

4.1.2.1 Toxicokinetics, metabolism and distribution

Numerous studies on the toxicokinetics following oral administration of DEHP have been performed in experimental animals whereas only a few studies on humans are available. Limited data are available concerning inhalation and dermal exposure. Studies using parenteral administration have also contributed to the understanding of the toxicokinetics.

The structures of the metabolites referred to in the text are given in **Table 4.25** in the toxicokinetic overall conclusion and named according to the widely accepted nomenclature of Albro (Albro et al., 1983).

Studies to compare the toxicokinetic behaviour of DEHP between humans, different strains of non-human primates, different strains of rats, mice, hamsters, guinea pigs, dogs, and miniature pigs have been performed in different studies (CMA, 1982b; CMA, 1983; CMA, 1984a; Short et al., 1987; Astill et al., 1986; ICI, 1982a; Shell, 1982; Rhodes et al., 1986; Lake et al., 1984b; Ikeda et al., 1980; Egestad et al., 1996 and Albro et al., 1982a; 1982b). There are also studies that compare the toxicokinetics after different routes of exposure (Rhodes et al., 1983; General Motors, 1982ab; Pollack et al., 1985a; Tanaka et al., 1975; Lindgren et al., 1982). In this section

of toxicokinetics the whole study is described once, at the first appropriate occasion, and the next time the study is mentioned with a reference to the description.

4.1.2.1.1 Oral

The metabolism and excretion of DEHP has been extensively studied in rats following oral administration. Other species in which the metabolism and excretion by the oral route have been studied in less detail include mice, guinea pigs, hamsters, non-human primates, and humans.

Humans

Two healthy male volunteers (47 and 34 years old) received 30 mg DEHP (> 99% pure) as a single dose or 10 mg/day of DEHP for 4 days (Schmid and Schlatter, 1985). In the single dose study, urine was collected every six hours for 48 hours after dosage and metabolites were isolated and identified by GC/MS. Urinary excretion of DEHP occurred mostly within the first 24 hours and a urinary elimination half-life of about 12 hours was estimated. 11% and 15% of the administered dose was eliminated in the urine of the two volunteers, respectively. A total of 12 metabolites were detected with the major metabolites being identified and quantified as MEHP (6.4 and 12.7% of the detected metabolites, in the two volunteers, respectively) and metabolites I (1.9 and 2.1%), IV (3.7 and 1.8%), V (25.6 and 33.8%), VI (24.0 and 19.7%), VII (5.3 and 4.0%), and IX (33.0 and 25.9%). The amount of the remaining 5 metabolites was less than 1%. About 35% of the metabolites were unconjugated in both volunteers. In the repeated dose study, urine was collected in 24 hour intervals until 48 hours after the last dose. Fifteen and 25% of the administered dose was eliminated in the urine of the two volunteers, respectively. Excretion of metabolites showed strong daily fluctuations. Based on the mean excretion of MEHP (9.6%), 5-oxo-MEHP (21.8%), 5-OH-MEHP (29.4%), and an assumed total urinary excretion of 25%, Koch et al. (2003a) have calculated conversion factors of 2.4% (MEHP), 5.5% (5-oxo-MEHP), and 7.4% (5-OH-MEHP) based on this study.

A volunteer was given a either a single oral dose of 213 mg or on another occasion repeatedly 70 mg/day for 3 days (210 mg) DEHP in 40% w/v ethanol solution (Bronsch, 1987, the study is poorly described). Urine was collected and the metabolites were characterised by GC/MS. Following the single dose, 31% (66 mg) of the dose was recovered as DEHP metabolites in the urine within 47 hours. More than 53% (approximately 30 mg) of the recovered dose was eliminated under 4 hours and 90% within 24 hours. MEHP was the principal metabolite (approximately 55%) identified. Twenty-one other different metabolites were detected in the urine. Of these, more than 99% of these were conjugated to ß-glucuronic acid. After repeated dosing the amount eliminate in urine was 27% (57 mg) with a similar elimination time as for the single dose.

Anderson et al. (2001) administered several ^{13}C-labelled phthalate diesters, including DEHP, to two groups of eight human volunteers. The low-dose group received 168-255 µg of each phthalate and the high-dose group received 336-510 µg of each phthalate (dibutyl-, DEHP, diisooctyl-, and benzylbutylphthalate). A third group of eight individuals served as control and received no phthalates. The compounds were administered in spiked margarine spread on toast as breakfast. 24-hour samples of urine were collected 1 day before dose, 1 day after dose and subsequently 2 and 6 days after the dose. The urine was hydrolysed and analysed by LC-MS (no details presented in the paper). The monoesters of DEHP and di-isooctylphthalate (DIOP) coeluted in the system. Since the origin of the ^{13}C-MIOP was not mentioned in the paper, the authors were contacted for further details. The ^{13}C-DIOP and ^{13}C-MIOP were synthesised from ^{13}C-phthalic acid and ^{13}C-phthalic anhydride, respectively, and an isooctanol with unknown

positions and extent of branching (Castle, personal communication). Thus, the branching of the administered ^{13}C-DIOP is unknown. Secondary metabolites of MEHP were not analysed for. The combined excretion of the two monoesters (MEHP and MIOP) corresponded to 14% and 12% of the combined dose of the diesters for the high- and low-dose groups, respectively. The study is well-done, and a mean excretion of 13% for the sum of these metabolites is apparent. However, the relative contribution of MEHP and MIOP to this peak is unknown, and a conversion factor of 0-26% can be deduced for MEHP based on this study.

One may assume similar rates of hydrolysis of DEHP and DIOP in the first step of the metabolic pathway (Lake et al., 1977). In the second step, however, one may suspect a faster further metabolism of MEHP than of MIOP based on the general rule of thumb that branching reduces metabolism (by reducing the accessibility of the enzymes). The degree and positions of branching of MIOP is unknown, but since the very fast further metabolism of MEHP occurs at the second last carbon (C-5) of the side-chain (without any branching nearby; the ethyl group is situated at the C-2 position) the branching of MIOP is likely to lead to a slower further metabolism than of MEHP. In fact, some 20 metabolites have been indicated for DEHP/MEHP, and Barr et al (2003) and Koch et al. (2003c) have shown that the urinary concentrations of the secondary metabolites of MEHP together are 8-14-fold higher than the concentration of MEHP. If the further metabolism of MEHP is faster than that of MIOP, a build up of MIOP in urine is likely while MEHP to a very high extent is metabolised further. If this would be the case, a smaller part of the 13% would be represented by MEHP. In the absence of metabolism data on DIOP/MIOP, this is a speculation, but it is based on sound scientific principles, and leads us to conclude that the uncertainty around this value of 13% makes it impossible to use it as the basis for a conversion factor for DEHP/MEHP. Furthermore, considering that the concentration of the two secondary metabolites of MEHP is 8-14 times higher than the concentration of MEHP, if 13% would be a correct value for MEHP, then the total conversion factor of these three metabolites together would be almost twice the given dose, and still, the other 10-20 minor metabolites known to occur in urine and the share known to be excreted in feaces (10% after an i.v. dose; Peck et al., 1982) are not considered. In discussions with the principle author of the Anderson study (2001), he has also expressed serious doubts concerning using his study as basis for the conversion factor (Castle, personal communication).

The time-course of DEHP metabolism and elimination in one human volunteer has been further investigated in a recent study by Koch et al. (2003d). A single oral dose of 48.1 mg deuterium-labelled DEHP (0.64 mg/kg bw) was administered to a male volunteer (the senior author of the paper, age 61, body weight 75 kg). DEHP was spiked into butter and administered on bread. By the use of deuterium-DEHP and the most modern technique (LC-LC/MS-MS), the results are very reliable even though they only represent one individual. The urinary excretion of MEHP, 5OH-MEHP, 5oxo-MEHP was monitored for 44 hours post-dosing and the serum levels was monitored for 8 hours post-dosing. Peak concentrations in serum were found in the sample taken two hours after dosage, with MEHP as the major metabolite. The half-time of all the three measured metabolites in serum was estimated to be less than 2 hours.

The excretion of DEHP metabolites in urine followed a multi-phase elimination pattern. After an absorption and distribution phase of 4 to 8 hours, the urine elimination pattern showed an initial half-time (8 to 16 hours post-dosing) of about 2 hours for all three metabolites. The second phase, beginning 14 to 18 hours post-dosing, showed a half-time of about 5 hours for MEHP but 10 hours for the secondary metabolites. The study shows that the secondary metabolites 5OH-MEHP and 5oxo-MEHP are the major metabolites of DEHP found in human urine at all time points following a single oral dose of DEHP and that the ratio between MEHP and the secondary metabolites varies over time. Thus, the ratios of MEHP to 5OH-MEPH + 5oxo-MEHP

varied over time from 1 to 4.9 during the first phase (8-16 hours post dose) to 1 to 14.3 during the second phase (16 to 24 hours post dose). In the last sample, taken 44 hours after the dose, the ratio was 1 to 74. This difference in elimination half-times has to be taken into account when DEHP ingestion are calculated based on either MEHP or the secondary metabolites. After 44 hours, 47% of the DEHP dose had been excreted in urine as the three measured metabolites. MEHP comprised 7.3% of the applied dose, 5OH-MEHP 24.7% and 5oxo-MEHP 14.9%. Thus, the ratio of excreted MEHP to 5OH-MEHP + 5oxo-MEHP was 1 to 5.4 in this individual, which appears lower than in the two recent studies on the general population (Koch et al., 2003b and Barr et al, 2003), where the ratio of MEHP to 5OH-MEHP + 5oxo-MEHP were 1 to 8.7 and 1 to 14.1, respectively.

Non-human primates

In a study of GLP-quality, male Cynomolgus monkeys (2 animals per group) received 100 or 500 mg/kg bw/day of unlabelled DEHP (99.8% pure) in corn oil by gavage for 21 days (Short et al., 1987 and Monsanto, 1988). On day 22 each monkey received a single dose of (carbonyl-^{14}C)DEHP (radiochemical purity > 97%) followed by three daily doses of unlabelled DEHP on days 23 to 25. Urine and faeces were collected at intervals on days 22 to 25 and then the animals were sacrificed. The percentages of (^{14}C)DEHP derived radioactivity in urine, faeces, and selected tissues (blood, liver, spleen, intestines, intestinal contents, fat, brain, kidneys, adrenals, testes, urinary bladder) were determined by liquid scintillation. Urine samples collected from 0-24 hours were analysed for metabolites of DEHP by normal and reversed phase HPLC. (^{14}C)DEHP derived radioactivity was detected in some tissues (liver and intestines) at the 500 mg/kg dose level, but represented less than 0.2% of the dose administered. The plasma concentration curves (AUC) for DEHP derived radioactivity for the first 48 hours was 133 and 283 ug-hr/ml at 100 mg/kg bw and 387 and 545 ug-hr/ml at 500 mg/kg bw. For the dose of 100 mg/kg bw, the two individual monkeys excreted 20 and 55% (20 and 55 mg) in the urine and 49 and 39% (49 and 39 mg) in the faeces. For the the 500 mg/kg bw dose, 4 and 13% (20 and 65 mg) in the urine and 69 and 56% (345 and 280 mg) in he faeces. This was measured within 96 hours but the majority was excreted within the first 24 hours after dosing, with most of the remainder being excreted during the next 24 hours.

DEHP derived radioactivity in 0-24 hour urine samples was resolved into at least 15 metabolites and identified as MEHP, phthalic acid, metabolites I, III, IV, V, VI, IX, X, XII, XIII, XIV, and unidentified fractions. Major metabolites were MEHP, phthalic acid, metabolites V, IX, X, and probably XII, however, a great variability between the two individuals in both dose groups was observed. Polar components, including possible glucuronides, made up only a small percentage of the urinary radioactivity. This study indicates a species difference in the metabolism of DEHP in rats and a nonhuman primate as two of the major metabolites identified in the urine of rats (metabolite I, the end-product of ß-oxidation of V; and metabolite VI, which is believed to be the proximate peroxisome stimulator in rodents) were minor metabolites in monkey urine.

The recoveries of the amount of DEHP derived radioactivity in the urine indicated that absorption at 500 mg/kg bw is equivalent to that at 100 mg/kg bw. This suggests that a dependent reduction in the absorption of DEHP from the intestinal tract of Cynomolgus monkeys (see Marmosets). However, the AUC is greater for 500 mg/kg bw than 100 mg/kg bw indicating that absorption is greater above 100 mg/kg bw than at 100 g/kg bw [though one would expect proportionally higher values but AUC for a longer time than 48 hours should be conducted for 500 mg]. The difference in none recovered radioactivity in the urine at these two different dose groups may depend on lose, or a higher degree of an alternative excretion pathway (e.g. hepatobilary excretion) and/or a higher degree of retention in the body at 500.

A comparative species differences in the metabolism of DEHP was studied after administration of a single oral dose of 100 mg/kg bw (carbonyl-^{14}C)DEHP (radiochemical purity > 97%) in corn oil by gavage to three male Cynomolgus monkeys, five male Fisher 344 rats and five groups of five male B6C3F1 mice at (CMA, 1982b; CMA, 1983; CMA, 1984a; Short et al., 1987 and Astill et al., 1986). The study was using a method equivalent to a guideline study and conducted according to GLP. Urine and faeces were collected at intervals of 12, 24, 48, 72, and 96 hours after dosing. Blood samples were taken from the femoral vein of monkeys at 2, 4, 8, 24 hours and just prior to sacrifice. All animals were killed around 96 hours after dosing for tissue collection (liver, stomach, intestines, intestine contents, gall bladder wash and bile). Concentrations of radioactivity in urine, faeces and blood were determined at the specified intervals and concentrations of radioactivity in selected tissues and other biological samples were determined by liquid scintillation at around 96 hours after dosing. Faeces samples collected from 0-48 hours were pooled for the monkeys, and urine samples collected from 0-24 hours were pooled for each species and were analysed for metabolites of DEHP by HPLC. Urinary metabolites were isolated and the major metabolites were analysed by GC/MS.

All three species excreted 30-40% of the dose in the urine (rats 32.9%, mice 37.3%, monkeys 28.2%), primarily during the first 12 hours for rats and mice and during the first 24 hours for monkeys. All three species excreted around 50% of the dose in the faeces (rats 51.4%, mice 52.0%, monkeys 49.0%), primarily during the first 24 hours for rats and mice and during the first 48 hours for monkeys. The rates and extent of urinary and faecal excretion varied widely among monkeys. DEHP was detectable in some tissues in all three species. The mean concentrations detected, with the exception of monkey liver and rat intestinal contents, were less than 1 μg/g. The highest concentrations were detected in liver, intestinal contents, and fat for monkeys, rats, and mice, respectively. Total recoveries of the radioactivity administered were 79 (68-91%), 87 (82-92%) and 90% (63-102%) for monkeys, rats and mice, respectively. Radioactivity in 0-24 hour urine samples were resolved into 13, 15, and 14 components in rats, mice, and monkeys, respectively. The components in urine were identified as MEHP (not detected in rat), phthalic acid, metabolites I, II (not detected in monkey), III (not detected in rat), IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. Major urinary components in rats were metabolites I, V, VI, and IX. Major urinary components in mice were MEHP, phthalic acid, metabolites I, VI, IX, and XIII, and in monkeys: MEHP, and metabolites V, IX, and X. In monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either absent or present in negligible quantities. Radioactivity in 0-48 hour monkey faecal extracts and in 0-24 hour rat and mouse faecal extracts were resolved into 11, 10 and 10 components in rats, mice and monkeys, respectively. The faecal components were identified as DEHP, MEHP, phthalic acid, metabolites I-IV, VI, VII, IX, X, XII, XIII (not detected in monkey), and XIV (not detected in mouse). DEHP was a major faecal component in all three species and MEHP a major faecal component in rats and mice.

Based on the amount of DEHP derived radioactivity recovered in the urine of Cynomolgus monkeys, rats and mice a similar degree of oral absorption of DEHP is indicated at a dose level of 100 mg/kg bw.

Two major species differences in the metabolism of DEHP in rat and mouse were observed as MEHP was a major component in mouse urine but was not detectable in rat urine; metabolite V was a major component in rat urine but a negligible component in mouse urine. Some overall similarities were observed in the metabolism of DEHP in monkeys and rats. In both species the MEHP formed by hydrolysis of DEHP was further metabolized via the ω-oxidation pathway, generating metabolites X, V, and I which collectively made up 34 and 44% of the radioactivity in the urine of monkeys and rats, respectively; and via the (ω-1)-oxidation pathway, generating

metabolites IX and VI which collectively made up 19 and 29% of the radioactivity in the urine of monkeys and rats, respectively. However, some overall differences in metabolism were also observed between the two species. MEHP was a relatively major component of monkey urine (11%) but was not detected in rat urine. Also in monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either absent or present in negligible quantities. Furthermore, MEHP was extensively converted to metabolite V in the monkey but, in contrast to the rat, further oxidation to metabolite I was negligible. Also metabolite I was a major component in mouse urine. It appears therefore that ß-oxidative metabolism of DEHP is a major pathway in rodents but not in monkeys.

The disposition of DEHP was studied in marmosets (Rhodes et al., 1983). Groups of three male marmosets received a single dose of (^{14}C-ring labelled) DEHP (radiochemical purity 97.5%) by the oral route (100 and 2,000 mg/kg bw), intravenously (100 mg/kg bw), and intraperitoneally (1,000 mg/kg bw) (Rhodes et al., 1983 and Rhodes et al., 1986). Urine and faeces were collected for seven days and the radioactive content determined. Tissue samples were removed 7 days after the administration.

Following intravenous administration approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion) indicating a 2 to 1 ratio between the urinary and biliary (faecal) routes of excretion in the marmoset. Around 28% of the dose remained in the lungs with minimal levels in other tissues. A much smaller proportion of the dose was excreted following intraperitoneal administration (10% in the urine and 4% in the faeces) in a similar 2 to 1 ratio. Around 85% of the dose remained as unabsorbed ^{14}C in the peritoneal cavity with minimal amounts in the tissues (0.6%). Following oral administration of 100 mg/kg bw. 20-40% of the dose were excreted in urine and around 25% in faeces, and following administration of 2,000 mg/kg bw around 4% and 84% were excreted in urine and faeces, respectively. Minimal amounts remained in the tissues (< 0.1%). This indicates that oral absorption of DEHP by marmosets is dose-limited at 2,000 mg/kg bw compared with 100 mg/kg bw. Dose dependent reduction in the absorption of DEHP from the intestinal tract of the marmoset (according to the authors the amount absorbed is more equivalent to that expected for a 150 to 200 mg/kg bw dose).

A comparative toxicokinetic study was carried out in marmosets (3 males and 3 females, 12-18 months) and Wistar derived albino rats (3 males and 3 females, Alderley Park Specific pathogen-free strain, 6-8 weeks). The animals were given (^{14}C ring labelled) DEHP (radiochemical purity 97.9%) at doses of 2,000 mg/kg bw daily by gavage for 14 days (ICI, 1982a; Shell, 1982 and Rhodes et al., 1986). Two samples of blood (0.5 ml) from each animal were taken during 0-8 hour period after dosing on day 1 and 14. The rats were bled via the tail vein and the marmosets via the femoral vein. Twenty-four hours after the final dose the animals were killed by inhalation of carbon dioxide/oxygen, and samples of blood (5 ml) were withdrawn from each animal via the vena cava. Excreta were collected for 24 hours after administration of the dose on days 6 and 13. Immediately after the blood samples were taken each animal was dissected and whole liver, kidneys and testes taken for radiochemical analysis. Radioactivity was measured by liquid scintillation spectrometry. The radiolabelled compounds in urine and faeces were analysed by TLC to determine the distribution between DEHP and its metabolites.

The uptake of radioactivity into the blood of rats was rapid and peaked after 2-3 hours (126 and 206 µg/g in males and females, respectively) following administration on day 1, and after 6 hours (368 and 475 µg/g in males and females, respectively) on day 14. On both days, blood levels did not decline significantly during the 8-hour sampling period, but 24 hours after dosing on day 14, the levels were 66 and 158 µg/g in males and females, respectively. Blood levels in marmosets were considerably lower. They peaked 1 hour after dosing (5 and 8 µg/g in males and

females, respectively) at day 1 and after 1 and 3 hours after dosing in males and females, respectively, on day 14 (13 µg/g each) and had not declined significantly after 24 hours.

After dosing on day 6, male rats excreted 83% (53% in urine and 30% in faeces) and female rats excreted 63% of the dose (39% in urine, 24% in faeces). Male marmosets excreted 69 (1% in urine and 64% in faeces) and female marmosets excreted 80% of the dose (2% in urine and 75% in faeces) after the same exposure period. After dosing on day 13 male rats excreted 97% (56% in urine and 41% in faeces) and female rats excreted 96% of the dose (52% in urineand 43.6 in faeces), while male marmosets excreted 62% (1% in urine and 59% in faeces), and female marmosets excreted 75% of the dose (1% in urine and 71 in faeces). The discrepancies between the sum of urine and faeces and the total is due to cage washing. Two radiolabelled compounds were present in rat faeces (analysed by TLC), one being identified as DEHP (42% of the radioactivity from TLC), the other more polar compound (57% of the radioactivity) was not identified. In the faeces of marmosets, 98% of the recovered radioactivity was identified as DEHP. The levels of radioactivity in blood, expressed as µg equivalents of DEHP per g of blood in males and females, were 0.3 and 0.5% of the daily dose, respectively, in rats one hour after administration on day 1. The corresponding levels for marmosets were 0.02 and 0.03%, respectively. The levels of radioactivity in blood 24 hours after administration on 14th day of exposure were 0.2 and 0.5%, respectively, for male and female rats. The corresponding values for marmosets were 0.03 and 0.06%, respectively. The very high faecal elimination and the low levels of radioactivity in urea, blood and tissues in marmosets compared with rats suggests, in agreement with the single dose study by Rhodes et al., (1983) that DEHP at 2,000 mg/kg bw was poorly absorbed, whereas the urinary elimination data for rats indicate that at least half the dose was absorbed. The study also shows that repeated administration of DEHP in both rat and marmoset did not modify the proportion of dose excreted. The tissue levels in liver and in kidney were generally higher in female rats compared to male rats (liver 216 and 286 µg/g, kidney 115 and 176 µg/g in males and females, respectively). The mean residue level in testes was lower (36 µg/g) than in other tissues. Tissue levels in marmosets were considerably lower than in rats (liver 29 and 47 µg/g, kidney 15 and 35 µg/g in males and females, respectively).

Rats

Sprague-Dawley

Sjöberg et al. (1985c) studied the kinetics of DEHP and MEHP in immature and mature Sprague-Dawley rats in two different studies. In one study (9-10 rats per group; 25, 40, or 60 days old on the day of dosing) were given a single dose of 1,000 mg/kg bw of DEHP (99% pure) in corn oil by gavage. Blood samples, 0.25 ml drawn from a jugular vein, were taken at 1, 3, 5, 7, 9, 12, 15, 24, and 30 hours after dosing. The area under the plasma concentration-time curve (AUC) and the elimination half-life was calculated. Detectable plasma concentrations of DEHP (> 2 µg/ml) were found only in some of the animals 1-7 hours after dosing. MEHP was detectable in all but five plasma samples (the 24- and 30-hour sample of two 60-day old rats, and the 30-hour sample of one 60-day old rat). The maximal plasma concentration (C_{max}) of MEHP generally appeared one hour after dosing, but in some 25-day old animals it was observed at 3-7 hours after dosing. No differences in C_{max} were observed between the different age groups. C_{max} ranged between 48 and 152 µg/ml, with a mean of 93 µg/ml. The mean AUC (0-30 hours) of MEHP of 25-day old rats (1,213 µg·hr/ml) was significantly higher than that of the 40- and 60-day old rats (611 and 555 µg·hr/ml, respectively). No significant differences in the mean plasma elimination half-life of MEHP were observed when comparing the different age groups. The mean plasma elimination half-lives of MEHP were 3.9, 3.1 and 2.8 hours, respectively for 25, 40 and 60 days old rats. The binding of MEHP to plasma proteins was 98% in all dose

groups. In a second experiment by Sjöberg et al. (1985c), the excretion of DEHP was studied in immature and mature Sprague-Dawley rats. Two groups of 6 rats which were 25 and 60 days old, respectively, on the day of dosing were given single doses of 1,000 mg/kg bw of (carbonyl-^{14}C)DEHP (99% pure) in corn oil by gavage. The urine was collected daily for three days. The cumulative excretion of radioactivity was 44 and 26% in 25- and 60-day old rats, respectively, within the first 72 hours after dosing. More than 85% of the urinary radioactivity appeared within the first 24 hours. No intact DEHP or MEHP was found in the urine when analysed by TLC.

To examine the plasma concentration time profiles of MEHP and metabolites V, VI and IX (structures of the metabolites are given in **Table 4.25**) after oral administration of DEHP, two separate experiments were performed by Sjöberg et al. (1986a). In the first experiment, a suspension of DEHP (purity not stated) in propylene glycol was given to five 35-day old male Sprague-Dawley rats in a dose of 2.7 mmol/kg bw. Blood samples drawn from one of the jugular veins 0.5, 1, 2, 3, 5, 7, 9, 12, 15, and 22 hours after dosing. In the second experiment, five rats were given daily doses of 2.7 mmol/kg bw of DEHP in propylene glycol for 7 days. After the final dose, blood samples were collected at the same time intervals as in the first experiment. The plasma concentrations of MEHP and the metabolites were determined by gas chromatography-electron impact mass spectrometry.

The plasma concentrations and mean AUC's of each of the MEHP-derived metabolites were considerably lower than those of MEHP both after single and after repeated administration. The maximal plasma concentrations (MEHP, 0.55 and 0.56 μmol/ml; metabolite IX, 0.15 and 0.09 μmol/ml; metabolite VI, 0.06 and 0.07 μmol/ml; metabolite V, 0.06 and 0.09 μmol/ml after single and repeated doses, respectively) and mean AUC's (MEHP, 5.15 and 3.44 μmol/ml; metabolite IX, 0.84 and 0.46 μmol/ml; metabolite VI, 0.44 and 0.41 μmol/ml; metabolite V, 0.39 and 0.43 μmol/ml after single and repeated doses, respectively) did not differ significantly between animals given single or repeated doses of DEHP. The mean elimination half-life of MEHP was significantly shorter in animals given repeated doses (1.8 hours) than in those given a single dose (3 hours).

The disposition kinetics of DEHP was studied in male Sprague-Dawley rats following single or multiple administration of DEHP by various routes (peroral by gavage: 2,000 mg/kg bw; intra-arterial: 100 mg/kg bw; intraperitoneal: 4,000 mg/kg bw) (Pollack et al., 1985a). The animals were given a single dose of 2,000 mg/kg bw of DEHP (purity not stated) in corn oil by gastric intubation. Blood samples were drawn over a 30-hour period. Thereafter, repetitive doses of DEHP were administered to the same animals once daily for 7 days whereafter blood samples were collected over a 48-hour period. The concentrations of DEHP and MEHP in whole blood were determined by high performance liquid chromatography (HPLC). After a single oral dose, DEHP was absorbed relatively rapidly with a peak blood concentration of DEHP observed at approximately 3 hours. Systemic bioavailability of DEHP was low, approximately 13%. Blood concentrations of MEHP were much higher than those of the parent compound after oral administration. The blood concentrations of DEHP following repeated dosing were similar to those observed after a single dose. Secondary increase in the concentration of DEHP in blood were observed following administration by all three routes. Following a single intra-arterial injection a large apparent volume of distribution and a high rate of clearance was observed for DEHP. A marked route-dependency in the formation of MEHP from DEHP was observed. Pharmacokinetic calculations revealed that approximately 80% of an oral dose of DEHP undergoes mono-de-esterification, as compared to only about 1% of the dose following either intra-arterial or intraperitoneal administration. Multiple intraperitoneal injections resulted in an apparent decrease in the rate and/or extent of DEHP absorption from the peritoneal cavity, while

no significant change in the peroral absorption of DEHP was observed. The difference in the MEHP to DEHP AUC ratio between peroral and intraperitoneal routes was still evident after multiple dosing.

DEHP and MEHP were secreted into the milk of lactating Sprague-Dawley (CD) rats when given 3 oral doses of 2,000 mg/kg bw/day of DEHP in corn oil by gavage on days 15-17 of lactation (Dostal et al., 1987a). Plasma collected 6 hours after the third dose contained virtually no DEHP but substantial amounts of MEHP (76 µg/ml). Milk collected 6 hours after the third dose contained 216 µg/ml DEHP and 25 µg/ml MEHP. A very efficient extraction mechanism for DEHP was suggested because of a high milk/plasma ratio (see Sections 4.1.2.6.1 and 4.1.2.10.1).

Male Sprague-Dawley rats (number not stated, 250-350 g) were given two doses of 100 mg (7-^{14}C) DEHP (purity not stated) or (7-^{14}C)MEHP (purity not stated) in corn oil by gavage, 24 hours apart (Albro et al., 1983). Urine was collected from the time the first dose was given until 24 hours after the second dose. Metabolites were isolated and analysed by HPLC and GC, and the profiles of radioactivity of the urinary metabolites were determined. Twenty metabolites were identified in the urine of rats (see **Table 4.25**). The metabolites identified in the urine of rats treated with either DEHP or MEHP were identical. No glucuronides or other conjugates were detected.

In a second experiment of the same study, (7-^{14}C)DEHP was given as a single dose to a rat (300 g) and the urine collected was freezed immediately. One week later the same rat was given a dose of (7-^{14}C)DEHP identical to that above and the urine was collected. Metabolites were isolated and analysed by HPLC and GC. The profiles of radioactivity of the urinary metabolites in the two different samples were qualitatively identical indicating, according to the authors, that the presence of any of the metabolites found was not due to further metabolism by bacteria in the urine.

According to the authors, previous studies of the metabolism in rats led to the suggestion that the enzymatic processes normally associated with ω-, (ω-1)-, α- and ß-oxidation of fatty acids could account for the known metabolites of DEHP found in the urine. Several metabolites of DEHP have been identified in the present study. Their formation requires that the initial hydroxylation process is less specific than fatty acid ω- and (ω-1)-oxidation are thought to be. Furthermore, it is necessary to postulate either that the aliphatic chain of MEHP can be oxidised at two sites simultaneously, or that oxidation products can be recycled for a second hydroxylation prior to excretion.

Adult male Sprague-Dawley rats (CD, 300-400 g; number not stated) were administered two doses of 200 µl (196 mg) (7-^{14}C)DEHP (> 99% pure) in corn oil by gavage, 24 hours apart (Albro et al., 1973). The urine was collected for 48 hours after the first dose was given. Metabolites in the urine were analysed by TLC and gas chromatography (GC) and characterised by infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy (MS). Five metabolites were identified in the urine. The metabolites identified correspond to phthalic acid and to metabolites I, V, VI, IX resulting from ω- and (ω-1)-oxidation of MEHP without attack on the aromatic ring. MEHP was not detected in the urine and phthalic acid amounted to less than 3% of the urinary metabolites. Conjugates were not detected. These results indicate, according to the authors, that DEHP is first hydrolysed to MEHP, which then undergoes ω- and (ω-1)-oxidation of the side chain. Alcohol intermediates may then be oxidized to the corresponding ketones. The metabolites found in the urine suggest that, in the rat, MEHP is metabolised like a fatty acid by ω- and (ω-1)-oxidation and then by ß-oxidation.

The absorption, disposition, and elimination of DEHP (99% pure) was also studied after administration of a single dose of 100 mg/kg bw of (2-hexyl-^{14}C)DEHP by gavage to four fasted male Sprague-Dawley rats (Eastman Kodak Co., 1983) The study was comparable to a guideline study and according to GLP. Urine, faeces, and expired air were collected for 6 days, thereafter the animals were killed by cervical dislocation. Radioactivity was measured in urine, faeces, expired air, organs and tissues (brain, heart, lungs, liver, spleen, kidneys, small and large intestines testes and samples of adipose tissue) by liquid scintillation spectrometry. Most of the radioactivity was excreted in the faeces (62%) with 34% as DEHP and 4% as MEHP (analysed by HPLC). The remainder of the radioactivity was eliminated in the urine (30%) as DEHP-metabolites and in the expired air as $^{14}CO_2$ (4%). Less than 2% of the dose was recovered in the carcass and tissues. All tissues except liver and abdominal fat had about the same concentration of radioactivity as was found in the carcass. Liver and abdominal fat values were about 6 and 4 times, respectively, that of carcass.

The elimination of DEHP was studied in rats and hamsters (Lake et al., 1984b). A single dose of (carbonyl-^{14}C)DEHP (> 99% pure) was administered to 5-week-old male Sprague-Dawley rats and male DSN strain Syrian hamsters at dose levels of 100 (5 rats, 3 hamsters) or 1,000 mg/kg bw (5 rats, 5 hamsters) in corn oil by gastric intubation. Urine and faeces were collected over a period of 96 hours and then the animals were sacrificed. Radioactivity was measured in urine, faeces, and total gut contents by liquid scintillation spectrometry. Faecal metabolites were extracted and chromatographed on thin-layer plates. In both species the bulk of the radioactivity was excreted within 24 hours. At the lower dose level, both species excreted more radioactivity in the urine (rat: 51%, hamster: 53%) than in the faeces (rat: 43%, hamster: 31%), whereas at the higher dose level, the major route of excretion was via the faeces (rat: 53%, hamster: 48%). In both species and at both dose levels, only negligible amounts of radioactivity were present at termination in either the liver, kidney, or total gut contents. Faecal radioactivity profiles were determined in 0-24 hour faeces samples. About 50% of the faecal radioactivity of rats at the higher dose level appeared to be the parent compound, the remainder comprised metabolites possibly including MEHP. In contrast, more than 95% of the faecal radioactivity of hamsters appeared to be the parent compound. Similar results were obtained with faecal extracts from rats and hamsters at the lower dose level.

Excretion and metabolism of DEHP were studied in a comparative study where DEHP (99.6% pure) was administered in the diet to adult male Sprague-Dawley rats (6 animals, 200-300 g), male beagle dogs (4 animals, approximately 1 year old, 7-10 kg), and male miniature pigs (5 animals, Hormel strain, between 4 month and 1 year, 10-25 kg) in doses of 50 mg/kg bw/day for 21-28 days before administration of a single dose of (carbonyl-^{14}C)DEHP (radiochemical purity > 98%) (50 mg/kg bw) in corn oil by gavage (Ikeda et al, 1980). Administration of the DEHP containing diets was continued until the animals were killed. Distribution and excretion of the radioactivity in urine, faeces, and various organs and tissues (liver, kidney, g.i.-tract with content, lungs, brain, fat and muscle) were analysed at various times by liquid scintillation. Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respectively; and after 4 days 37 and 53% (rats), 21 and 75% (dogs), and 79 and 26% (pigs), respectively. Elimination of radioactivity was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four radioactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolised DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 in all species and a small amount remained after 4 days. In other organs there was only a small amount of radioactivity present in all samples. Of the remaining organs the highest level (about 2% of the dose) was found in the livers from rats after 4 hours.Bile samples from dogs, and to a lesser

extent from pigs, accounted for a significant amount of administered ^{14}C dose. Less than 1% of the administered ^{14}C dose was secreted in the bile from bile duct cannulated rats.

Metabolism and tissue distribution of mono-2-ethylhexyl phthalate (MEHP) has been studied in male Sprague-Dawley rats (Chu et al., 1978). To study if MEHP was readily absorbed orally, the carotid arteries of 8 rats were cannulated and 4 days later 4 animals were given 69 mg (7-^{14}C)MEHP/kg (20 μCi) in corn oil via stomach tube. Serial blood samples (0.2 ml) were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours after dosing and radioactivity was determined. The blood level was highest in the first sample, after 0.5 hours, and then rapidly decreased. About five hours after dosing there was a small increase in the bloodconcentration in all animals whereafter the concentration slowly continued to decrease.

Another four rats were given 35 mg (7-^{14}C)MEHP/kg in 5% $NaHCO_3$ via the cannula. Serial blood samples were collected at 2, 4, 6, 8, 12,, 14, 16, 18 and 20 minutes after the injection and analyzed for radioactivity. Immediately after the blood samples were taken the animals were exsanguinated and tissues and organs were removed for determination of radioactivity. The rapid decrease was observed also in the rats administered i.v. The first blood sample showed that 53% of the radioactivity remained in the blood. Approximately 1/3 and 1/5 of the radioactivity was retained in the blood 10 and 20 min after administration, respectively. Twenty minutes after an i.v. dose the liver, bladder and kidney were found to possess high radioactivity, but other tissues also had some radioactivity. One of the major deposition sites for radioactivity shortly after i.v. injection was the liver.

In a third experiment two groups of four rats were given 69 mg (20 μCi) and 6.9 mg (2 μCi) (7-^{14}C) MEHP/kg, respectively, in corn oil via stomach tube. The animals were exsaguinated after 24 hours and tissues and organs were removed for determination of radioactivity. Twenty-four hours after an oral dose of 69 mg (7-^{14}C) MEHP virually all radioactivity was removed from the body and only traces were present in the kidney, liver, heart, lung, intestine and muscle. No detectable amounts could be found in the tissues of rats 24 hours after an oral dose to 6.9 mg/kg.

In a fourth study four rats were given a single oral dose of 69 mg (7-^{14}C) MEHP/kg (20 μCi) in corn oil via stomach tube, and were kept individually in metabolism cages. Urine and faeces were collected each day for 7 days for radioactivity measurements. Only the urine was examined for metabolites as this route of excretion accounted for 81% of the dose. Excretion after 48 hours was insignificant. At least four metabolites were identified and they had previously been identified as DEHP metabolites.

The bile ducts were cannulated in four rats in a fifth study by the same author. These rats were given 3.5 or 35 mg (7-^{14}C) MEHP/kg in 5% $NaHCO_3$ via the superficial dorsal vein of the penis. Serial bile samples were taken hourly for 8 hours and assessed for radioactivity. Within 8 hours 52% (3.5 mg/kg) and 40% (35 mg/kg) of the dose was secreted, respectively, and secretion after this time was insignificant.

Thus, the present study indicate that MEHP given orally to the rat undergoes ω- and (ω-1)-oxidation to yield the same metabolites as does DEHP, and suggest that MEHP is the intermediary product in the DEHP metabolism. More than 80% of the radioactivity of the orally administered (7-^{14}C) MEHP was excreted in urine within 24 hours. Up to 52% of the radioactivity entered the intestine from the bile whereas only 8% of the dose was excreted in faeces. This would indicate that resorption of radioactivity took place in the intestine. The rise in the radioactivity in the blood after the rapid decrease could be attributed to the reabsorption of biliary secreted material. The percent radioactivity secreted in the bile was lower, 40%, at the higher exposure level (35 mg/kg) compared to 52% at the lower exposure level (3.5 mg/kg).

Wistar

The distribution and elimination of DEHP and MEHP after a single oral dose of 25 mmol DEHP/kg (corresponding to 9765 mg/kg bw, purity not stated) by gastric intubation were studied in male JCL:Wistar rats (number not stated, 200 g) (Oishi and Hiraga, 1982). Samples of blood and tissues were collected at 1, 3, 6, 24, 48 and 96 hours post-intubation, and analyzed by gas-liquid chromatography and a electron capture detector. The concentration of DEHP and MEHP in blood and tissues increased to a maximum within 6-24 hours after dosing while the highest levels observed in the heart and lungs occurred within one hour. Both DEHP and MEHP were detected in brain and kidney, but the concentrations were very low. Only small amounts of MEHP was measured in the lung, and DEHP was detected in the spleen at very low levels. The concentration of DEHP in fat increased gradually until 48 hours after dosing. The concentration of DEHP in liver declined with a half-life of 1 day while that in the epididymal fat declined more slowly with a half-life of 6.5 days. At 6 hours after administration, the highest ratio of MEHP/DEHP was recorded in testes (2.1). The ratio in blood was 1.1 while the ratio in other tissues was less than one. Biological half-lives of DEHP in different tissues ranged from 8 to 156 hours in the testicular tissue and epididymal fat, respectively, and of MEHP from 23 to 68 hours in the blood and epididymal fat, respectively.

A more recent study by Oishi (1990) reported on the distribution and elimination of DEHP after a single oral dose of DEHP (2,000 mg/kg bw) in male Wistar rats (35 days old). The blood was collected from the caudal vena cava under deep ether anestesia and then testes were removed at 1, 3, 6, 12 and 24 hours following DEHP administration. The concentration of MEHP in blood and in testis increased to a maximum 6 hours after administration of DEHP and then slowly decreased. For MEHP the biological half-lives in blood and testis were 7.4 and 8.0 hours, respectively, and the area under the concentration-time curve was 1,497 and 436 μg·h per ml or per g, respectively.

Young male Wistar rats (number not stated, 100-200 g) were treated with a single dose of (carbonyl-^{14}C)DEHP (purity not stated) at a dose level of 2,000 mg/kg bw in corn oil by gastric intubation following pre-treatment with DEHP (> 99% pure) for 0, 6 or 13 days (Lake et al., 1975). At the end of 4 days, when no further radioactivity was detected in the excreta, the animals were sacrificed, and the organs and tissues were removed. The radioactivity in excreta, organs, and tissues were measured by liquid scintillation spectrometry. Following a single dose of DEHP, virtually all of the administered radioactivity was excreted in the urine (52%) and faeces (48%) within 4 days, and less than 0.1% of the radioactivity remaining in organs and tissues. Similar results were observed in rats pre-treated with DEHP for 6 or for 13 days (60% of the radioactivity was recovered in urine and 40% in faeces).

In several experiments by Tanaka et al., (1975), male Wistar rats (150-250g) were given single oral doses (500 mg/kg bw) or a single intravenous doses (50 mg/kg bw) of (carbonyl-^{14}C) DEHP (radiochemical purity > 99%) to study distribution, metabolism and elimination. In the elimination studies there were two animals in each group, and in the distribution studies there were three animals in each. The peak blood level was observed about 6 hours after administration. The concentrations in liver and kidney reached a maximum in the first 2-6 hours. No significant retention was found in organs and tissues (brain, heart, lungs, liver, spleen, kidney, stomach, intestine, testicle, blood, muscle an adipose tissue). About 80% of the dose was excreted in the urine and faeces within 5-7 days following both oral and intravenous administration. Excretion in the urine was generally slightly greater than that in the faeces. In experiments with rats in which the bile duct was cannulated, about 5% of the dose was recovered from the bile in 24 hours after oral administration, whereas about 24% was recovered after intravenous administration. When urine and faecal extracts were analysed by thin-layer

chromatography (TLC) after oral administration, four major metabolites were detected in urine. Unchanged DEHP was excreted in the faeces, but DEHP or MEHP were not detected in the urine or bile. After intravenous administration about 75% of the dose was recovered from the liver after the first hour. The radioactivity of the liver declined rapidly by about 50% within the next 2 hours and only 0.17% of the radioactivity remained on the 7th day. The intestine accumulated the next highest amount of radioactivity. The radioactivity increased as the radioactivity in the liver decreased. After intravenous application the activity in the liver and kidneys reach a maximum in the first 2-6 hours. Medium values were seen in the heart, lung and spleen. The peak blood level was observed about 6 hours after administration. The testicle and brain showed the lowest values as in the case of intravenous application.

The distribution, accumulation, and excretion of DEHP were studied in several experiments with Wistar rats (Daniel and Bratt, 1974). Following a single oral dose of (carbonyl-^{14}C)DEHP (2.9 mg/kg bw; purity not stated), rats (5 adult males) excreted 42% and 57% of the administered radioactivity in the urine and faeces, respectively, within 7 days. Rats (5 adult males) fed a diet containing 1,000 ppm of DEHP 7 days prior to dosing with (carbonyl-^{14}C) DEHP excreted 57% and 38% in the urine and faeces, respectively, within 4 days.

In studies with biliary-cannulated rats, administered 2.6 mg/kg DEHP by intubation, around 10% was excreted in the bile.

In rats (24 females) fed a diet containing 1,000 or 5,000 ppm of (carbonyl-^{14}C)DEHP for 35 and 49 days, respectively, the amount of radioactivity in liver and abdominal fat rapidly attained a steady-state concentration without evidence of accumulation. When returned to a normal diet, the radioactivity in the liver declined with a half-life of 1-2 days and in fat with a half-life of 3-5 days. DEHP was extensively metabolized with 14 metabolites, including MEHP, present in urine (analysed by TLC, MS and NMR). DEHP was not detected in the urine. The principal metabolites detected correspond to phthalic acid and metabolites IV or V, VI and IX i.e. the acid, alcohol, and ketone resulting from ω- and (ω-1)-oxidation of MEHP. The hexobarbital sleeping time was reduced 39 and 43% in male and females, respectively, when given five daily oral doses of DEHP. When DEHP was administered intravenuosly the hexobarbital sleeping time increased by approximately 40% in male rats compared with the corresponding controls.

Rats were injected 600 mg ^{14}C-DEHP/kg as an emulsion prepared by subjecting to ultrasonication through the femoral vein. After 2, 24, 72 and 96 hours the animals were killed and the lungs, liver, spleen, blood and portions of abdominal fat were removed for radiochemical analysis. Radioactivity disappeared rapidly from the blood and about 60-70% was recovered in the liver and lung within 2 hours of dosing. After 4 days 44% was recovered from the urine, 29% from the faeces. About 1% was recovered in fat.

Lhuguenot et al. (1985) studied the metabolism of DEHP and MEHP in rats following multiple dosing. Adult male Wistar rats (180-220 g, 3 rats per group and per chemical) were administered (7-^{14}C)DEHP (> 98% pure) or (^{14}C)MEHP (position of label not stated; highest available purity) by gastric intubation in corn oil at doses of 50 or 500 mg/kg bw for three consecutive days. Urine was collected for 4 days, at 24-hour intervals, metabolites were extracted, analysed by GC and detected by MS. After exposure to DEHP approximately 50 and 60% and after exposure to MEHP approximately 70 and 80% of the total daily doses were recovered in the urine at the low and high dose levels, respectively. No water-soluble conjugates were detected in the urine following administration of DEHP or MEHP. After a single dose of either compound, the main metabolites excreted were I, V, VI, IX. At the lower dose level, no or minor changes in urinary metabolite profiles were seen with time; after multiple dosing at the higher dose level, increases

in ω-/β-oxidation products (metabolites I and V) and decreases in (ω-1)-oxidation products (metabolites VI and IX) were seen.

Fischer 344

Male Fischer 344 rats (12 animals per group) received a total of 10 daily doses of 1.8, 18, or 180 mg/kg bw of radiolabelled DEHP (purity not stated) in cottonseed oil by gavage. All rats received the same amount of radioactivity (1.8 mg/kg bw (^{14}C)DEHP, position of label not stated) with different amounts of non-radioactive DEHP diluent (Albro et al., 1982a). Urine and faeces were collected daily. Three rats from each group were sacrificed 1, 3, 10, or 12 days after receiving their first dose of DEHP. Various tissue samples and faeces were radioassayed using a tissue oxidizer; urine was radioassayed directly in liquid scintillation fluid. The profiles of the radioactive metabolites in urine were determined by HPLC. Unhydrolysed DEHP was measured by Radio-TLC. The percentage of ^{14}C retained in the liver tended to decrease with exposure time and also with increasing dose. There was no evidence for accumulation of DEHP in the liver. Essentially the same observations applied to the testes except that testes had lower concentrations than the liver. After about 4 days, excretion (cumulative excretion of ^{14}C as a percentage of the cumulative dose) became quite independent of the dose. Up to a dose of 180 mg/kg per day there was no indication of beginning to saturate the overall elimination mechanism.

In a separate experiment, rats and mice were given single oral doses of DEHP in cottonseed oil by gavage at doses ranging from 1.8 to 1,000 mg/kg bw. The animals were sacrificed 6 hours later and the livers were assayed for intact DEHP as described above. In Fisher rats, as the dose increased, a threshold was reached, at about 450 mg/kg bw, above which there was a steady increase in the amount of unhydrolysed DEHP reaching the liver. According to the authors, intact DEHP will reach the liver of rats whenever its concentration exceeds 0.43% in the diet. In contrast, an absorption threshold could not be determined in either CD-1 or C3B6F$_1$ mice for doses up to 1,000 mg/kg bw. According to the authors, this may reflect the higher level of DEHP-hydrolase in the intestines of mice than in rats. Preliminary expeiments with both Sprague-Dawley (CD) and Fischer 344 rat revealed that the maximum amount of DEHP that could be given as a single oral dose without significant excretion of unabsorbed DEHP in the faeces was 200 mg/kg bw. According to the authors, pharmacokinetic studies using 14C-labelled DEHP at doses above 200 mg/kg bw would rapidly become dominated by unabsorbed DEHP and, according to the authors, could not be directly compared to rats of elimination at lower doses.

Male Fischer 344 rats (100-150 g bw, 12 animals per group) were fed diets containing 1,000, 6,000, or 12,000 ppm (estimated to correspond to 85, 550 and 1,000 mg/kg bw/day, respectively) of non-radiolabelled DEHP (99.8% pure) (CMA, 1982a; Lington et al., 1987; Short et al., 1987 and Astill et al., 1986). These groups were divided into three subgroups each consisting of 4 rats which received diets for 0, 6 or 20 days, followed by a diet containing a similar level of (carbonyl-^{14}C)DEHP (radiochemical purity > 97%) for 24 hours. Urine and faeces were collected at 24-hour intervals from 24-96 hours after administration of radiolabelled DEHP and then the animals were sacrificed. Four animals from each dose group were sacrificed on each of days 5, 11 and 25 and terminal blood samples were collected. Liver, lung, spleen, intestines, fat, brain, kidney, adrenals, testes and urinary bladder were removed. Urine samples collected from 0-48 hours were pooled for each of the three dose levels and three prior exposure regimens and analysed by normal and reverse phase HPLC for metabolites of DEHP. Urinary metabolites were isolated and the major metabolites were analysed and identified by GC-MS. Faeces samples

were similarly pooled and analysed by normal HPLC for the metabolites of DEHP. Radioactivity was measured by liquid scintillation.

At all dose levels and exposure times radioactivity was excreted primarily via the urinary route and primarily during the first 24 hours. The percentage of the dose excreted in urine increased with dose, from 53% at 1,000 ppm to 62-66% at 6,000 ppm, and 66-69% at 12,000 ppm. The faecal excretion occurred primarily during the second 24 hours. The percentage of the dose excreted in faeces decreased with dose, from 35-38% at 1,000 ppm to 26-30% at 6,000 ppm and 24-28% at 12,000 ppm. At all dose levels, prior exposure to DEHP did not affect the extent or rate of urinary or faecal excretion. Less than 1% of the administered dose remained in tissues 4 days after treatment. The radioactivity in the urine samples was resolved into 14 components and identified as phthalic acid, metabolites I, II, III, IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. The major urinary metabolites, I and V, were followed by metabolite IX and phthalic acid; the remainders was all present in only minor quantities. DEHP and MEHP were not detected in the urine. The radioactivity in the faeces sample was partially resolved into 15 components and tentatively identified as DEHP, MEHP, phthalic acid, metabolites I-V as a pool, metabolites VI, VII, IX, X, XII, XIII, and XIV. The major faecal metabolites were MEHP, metabolites I-V, VI and IX.

The urinary, and to a lesser extent the faecal, metabolite excretion patterns changed with dose and prior exposure to DEHP. The major changes in metabolism occurred between the 1,000 and 6,000 ppm dose levels and between 0 and 6 days prior exposure to DEHP. Minor changes were observed with increase in dose to 12,000 ppm. Urinary elimination of metabolites I and V were measured as a function of dose and duration of treatment. The output of metabolite 1 was relatively constant at all dietary levels on day 0, while the output of metabolite V increased with dietary level on day 0. Following prior exposure to DEHP at the 1,000 ppm dose level the urinary excretion of metabolite I doubled compared with no prior exposure, while the the urinary excretion of metabolite V remained relatively constant. Following prior exposure to DEHP at the 6,000 or 12,000 ppm dose level the urinary excretion of metabolite I increased three or four times, respectively, compared with no prior exposure. The urinary excretion of metabolite V decreased two or three times, respectively, following prior exposure to DEHP at the 6,000 or 12,000 ppm dose level compared with no prior exposure. According to the authors the increase in urinary levels of metabolite V at 6,000 ppm and higher while metabolite I remained relatively constant, indicated that at high exposure levels the initial dose of DEHP exceeded the rats ability convert metabolite V to metabolite I. However, the capacity for β–oxidation appeared to increase with repeated exposure to DEHP since urinary levels of metabolite V decreased with subsequent doses of DEHP while those of metabolite I increased.

The tissue distribution was examined in rats sacrificed 112-116 hours after receiving the radiolabelled DEHP. A major source of radioactivity was found in the intestinal contents. Additional sources of radioactivity included the liver, fat, kidney and adrenals. A comparison of the tissue levels of radioactivity after 0 and 20 days of pretreatment, indicated that pretreatment with DEHP for 20 days did not significantly alter the tissue distribution of ^{14}C-DEHP derived radioactivity.

To study peroxisome proliferation Fisher 344 rats (5 males/group) were fed diets containing 100, 1,000, 6,000, 12,000, 25,000 ppm DEHP, corresponding to 11, 105, 667, 1,223 and 2,100 mg DEHP/kg bw/d (Short et al., 1987). After an overnights fast the rats were sacrificed and their livers were examined for parameters indicative of peroxisome proliferation. Liver weight expressed as percent of body weight was significantly increased in rats that received 667 mg/kg bw/d and above. Palmitoyl-CoA oxidation and lauric acid 11- and 12-hydroxylation weight was significantly increased in rats that received 667 and 105 mg/kg bw/d, respectively, and above.

Since an increased peroxisomal score was observed at dose levels that also produced significant changes in the biochemical parameters, these observations appear to be correlated.

Fischer 344 rats, CD mice, Syrian golden hamsters, and Hartley albino guinea pigs were given two doses of (carbonyl-^{14}C) DEHP (purity not stated) in cottonseed oil by stomach tube at 24 hours intervals (Albro et al., 1982b). The maximum single dose of DEHP was 180 mg/kg bw for rats and guinea pigs, 360 mg/kg bw for mice, and 20 mg/kg bw for hamsters. Urine was collected for a total of 48 hours following the first dose. The urinary metabolites were analysed by HPLC and GC-MS. Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. **Table 4.26** gives the distribution of radioactive metabolites in pools of urine from three animals, however, it should be noted that the doses were not the same between species. No components were detected in urine from hamsters or guinea pigs that were not also present in urine from rats and mice. In the hamsters the main metabolites were: di methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating metabolite. MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the species tested. **Table 4.27** gives the forms of excretion products in urine.

DEHP was detected in foetal livers when pregnant albino rats were given daily doses of 1,000 mg/kg bw of DEHP in corn oil by gavage from day 6 to 15 of gestation, indicating that DEHP can cross the placental barrier (Srivastava et al., 1989) (see also Section 4.1.2.10.1).

Parmar et al. (1985) studied the effects on rat pups from dams (strain not stated) given DEHP through the lactation period. Pups from 10 litters were pooled and seven pups were randomly assigned to each mother. Five mothers were given 2,000 mg/kg bw of DEHP (vehicle not stated) daily by oral gavage from day 1 of birth up to day 21, and five mothers served as control group and was given saline. DEHP was detected in the livers of pups from treated mothers indicating that DEHP can be transferred through the milk (see also Section 4.1.2.10.6).

The metabolism of 2-ethylhexanol (2-EH), a metabolite of DEHP, was studied in two adult male rats administered (1-^{14}C)ethylhexanol (purity not stated) in cottonseed oil by gavage (Albro, 1975). Carbon dioxide (from expired air), urine, and faeces were collected at hourly intervals for 28 hours after administration. Metabolites in the urine were identified by GC and MS. 2-EH was efficiently absorbed and radioactivity from 2-EH was rapidly excreted in respiratory carbondioxide (6-7%), faeces (8-9%), and urine (80-82%), with essentially complete elimination by 28 hours after administration. Other metabolites identified were 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid and 2-ethyl-1,6-hexandioic acid. Only about 3% of 2-EH was excreted unchanged. Thus, these data indicate that the carbon chain of 2-EH is ultimately metabolized through oxidation pathways (ω- and (ω-1)-oxidation with subsequent ß-oxidation) to acetate and carbon dioxide.

Mice

Male C57BR mice (10-12 g, three groups of one control animal and 8 exposed) were given a single oral dose of 6.72 mg (carbonyl-^{14}C)DEHP (radiochemical purity >98%) by gavage. The animals were killed after 1, 2, 4, 8, 24 hours and 3, 5, 7 days, respectively, and were examined by whole-body autoradiography (Gaunt and Butterworth, 1982). Following absorption, the radioactivity was widely distributed in organs and tissues without evidence of tissue storage. The contents of stomach and small intestine showed marked evidence of radioactivity in all mice

during the first 24 hours, but only a slight reaction of one animal was recorded on day 3. Radioactivity was present in the caecal contents at 1 hour, increased to a maximum at 2 hours and persisted for 1 day, but was found only in one animal on day 3. No radioactivity was detected in the colon contents or faeces after 1 hr the activity reach a maximum at 2 and 4 hours in colon contents and faeces, respectively. The decline in radioactivity was similar to that in other parts of the gastro-intestinal tract. In the bladder there was a high level of activity between 1-24 hours and some activity in 2 of 3 mice on day 3. In the kidney activity in the parenchyma was similar to that in many tissues of the same animal, but it was more concentrated in the renal pelvis and papillae. Radioactivity in the testis was obvious only in one animal (killed after 4 hours) and was similar to the general tissue levels. In other tissues the level of radioactivity varied considerably between animals even at the same examination interval.

The distribution and tissue retention of DEHP following intravenous and oral administration was studied in mice with whole body autoradiography (Lindgren et al., 1982). In one experiment two male C57BL mice received 10 μCi of (2-ethylhexyl-1-^{14}C) DEHP (chemically and radiochemical purity > 99%) intravenously, corresponding to 9.6 mg/kg bw of DEHP. Another 2 male mice were given 10 μCi (carbonyl-^{14}C)DEHP intravenously, corresponding to 3.6 mg/kg bw of DEHP. The distribution was similar following administration of either substance. Four hours after intravenous injection, a very high activity was observed in the gall bladder, intestinal contents and urinary bladder. A high uptake was also seen in the liver, kidney, and brown fat. Some activity was observed in the white fat, myocardium, and muscles. The level in blood, bone, cartilage, testes, and nervous system was very low. Twenty-four hours after administration the activity in the gall bladder, intestinal contents and urinary bladder was still very high. The concentration in brown fat was high, but the activity in the liver and kidney was lower than after 4 hours.

In a second experiment of the same study (Lindgren et al., 1982), the effects of pre-treatment on the distribution of (^{14}C)DEHP were studied in male mice by whole body autoradiography. Four mice were given DEHP (10 mg/kg) by oral intubation once daily for 5 consecutive days, 2 mice were given daily intraperitoneal injections of phenobarbital sodium (75 mg/kg/day) in physiological saline for 3 consecutive days, and another 2 mice were intraperitoneally treated with 3-methylcholantrene (30 mg/kg/day) for 4 consecutive days. Twenty-four hours after the last administration the animals received 10 μCi DEHP in 20 μl ethanol (corresponding to 9.6 mg DEHP/kg for *2-ethylhexyl-1-^{14}C* and 3.6 mg DEHP/kg for *carbonyl-^{14}C*). The mice were killed 24 hours after injection with (^{14}C) DEHP. Following pretreatment of male mice with either DEHP, phenobarbital sodium or 3-methylcholantrene, the distribution 24 hours after the injection of either (carbonyl-^{14}C) DEHP or (2-ethylhexyl-1-^{14}C) DEHP was similar to that in the non-pre-treated animals, except that the concentration in the brown fat was higher in all the pre-treated animals as compared to non-pre-treated animals.

In a third experiment of the same study Lindgren et al. (1982), six pregnant mice were each given 10 μCi (^{14}C)DEHP in soy bean oil by oral intubation. The mice were killed after 4 and 24 hours. Mice at gestation day 8 were given 7.7 mg DEHP/kg for *2-ethylhexyl-1-^{14}C* and 2.9 mg DEHP/kg for *carbonyl-^{14}C,* and mice at day 16 of gestation received 4.8 mg DEHP/kg for *2-ethylhexyl-1-^{14}C* and 1.8 mg DEHP/kg for *carbonyl-^{14}C*. Whole body autoradiography was performed as described previously. After the pregnant mice had been killed the uteris were removed with their embryos, except for a few fetuses from mice in the late stage of pregnancy which were removed surgically from the uterus. As for the uteris, the maternal livers and kidneys were also removed for autoradiography. At early gestation marked uptake was seen in the yolk sac. There was a high concentration in the gut of the embryo 4 hours after administration of (carbonyl-^{14}C) DEHP on gestation day 8. On gestation day 9, 24 hours after administration of

(2-ethylhexyl-1-^{14}C) DEHP pronounced activity was observed in the neuroepithelium of the embryos. A high concentration was also seen in the uterine fluid. Except for the gut and the neuroepithelium, uptake in the embryo was low. In the mice at late gestation, a very high accumulation was seen in the yolk sac after oral administration of either (2-ethylhexyl-1-^{14}C) DEHP or (carbonyl-^{14}C) DEHP. The distribution in the fetuses was very similar after administration of the two ^{14}C-labelled DEHP compounds. Four hours after administration on gestation day 16 there was high activity in the renal pelvis, urinary bladder and intestinal contents. Some activity was seen in the skeleton and liver. On gestation day 17 there was little activity left in the fetuses, although a rather high activity was observed in the renal pelvis, urinary bladder and intestinal contents.

The distribution and retention of DEHP was studied in the NMRI mouse brain and liver (Eriksson and Darnerud, 1985). (7-^{14}C)DEHP (0.7 mg/kg bw; purity not stated) was administered by gavage to young mice (3-20 days old). One and 7 days after treatment the amount of radioactivity in the liver and brain was measured. The amount of radioactivity in the brain was low, especially in 10- and 20 days-old mice, and retention of radioactivity in the brain was minimal. The amount of radioactivity in the liver was about 10 times that in the brain. After 24 hours the amount of radioactivity found in the livers ranged from about 27 to 2% in the order 3-, 10- and 20-day-old mice, showing significant decreases in all ages after 7 days.

Male CD mice, guinea pigs, rats, and hamsters were given two doses of (carbonyl-^{14}C) DEHP (360 mg/kg bw for mice) by stomach tube at 24 hours intervals to compare the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982b). The study is presented in the section on oral administration to Fischer 344 rats. Rats predominately excreted metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. **Table 4.26** gives the distribution of radioactive metabolites in pools of urine from three animals, however, it should be noted that the doses were not the same between species. No components were detected in urine from hamsters or guinea pigs that were not also present in urine from rats and mice. In the hamsters the main metabolites were: di-methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating nmetabolite. MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the species tested. **Table 4.27** gives the forms of excretion products in urine.

Studies to compare the toxicokinetic behaviour of DEHP between male B6C3F1 mice, rats and Cynomolgous monkeys has been performed (CMA, 1982b; CMA, 1983; CMA, 1984a; Short et al., 1987 and Astill et al., 1986) and is presented in the section on oral administration tonon-human primates.

All three species excreted 30-40% of the dose in the urine (rats 32.9%, mice 37.3%, monkeys 28.2%), primarily during the first 12 hours for rats and mice and during the first 24 hours for monkeys. All three species excreted around 50% of the dose in the faeces (rats 51.4%, mice 52.0%, monkeys 49.0%), primarily during the first 24 hours for rats and mice and during the first 48 hours for monkeys. The rates and extent of urinary and faecal excretion varied widely among monkeys. DEHP was detectable in some tissues in all three species. The mean concentrations detected, with the exception of monkey liver and rat intestinal contents, were less than 1 μg/g. The highest concentrations were detected in liver, intestinal contents, and fat for monkeys, rats, and mice, respectively. Total recoveries of the radioactivity administered were 79 (68-91%), 87 (82-92%) and 90% (63-102%) for monkeys, rats and mice, respectively. Radioactivity in

0-24 hours urine samples were resolved into 13, 15, and 14 components in rats, mice, and monkeys, respectively. The components in urine were identified as MEHP (not detected in rat), phthalic acid, metabolites I, II (not detected in monkey), III (not detected in rat), IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. Major urinary components in rats were metabolites I, V, VI, and IX. Major urinary components in mice were MEHP, phthalic acid, metabolites I, VI, IX, and XIII, and in monkeys: MEHP, and metabolites V, IX, and X. In monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either absent or present in negligible quantities. Radioactivity in 0-48 hours monkey faecal extracts and in 0-24 hours rat and mouse faecal extracts were resolved into 11, 10 and 10 components in rats, mice and monkeys, respectively. The faecal components were identified as DEHP, MEHP, phthalic acid, metabolites I-IV, VI, VII, IX, X, XII, XIII (not detected in monkey), and XIV (not detected in mouse). DEHP was a major faecal component in all three species and MEHP a major faecal component in rats and mice.

In mice (strain and number not stated) given a single oral dose of 400 mg/kg bw of (^{14}C) MEHP (radiochemically pure, position of label not stated) in corn oil, the major metabolites (identified by GC/MS) in urine were recovered in the form of glucuronides (Egestad and Sjöberg, 1992). Three new metabolites were isolated and characterised as conjugates of ß-glucose. Thus glucosidation has been shown to be an alternative conjugation pathway, although less important.

A single oral dose of 400 mg/kg bw of (carbonyl-^{14}C) MEHP in corn oil was given to 11 male mice (strain not stated) and male guinea pigs (Dunkin Hartley, number of animals not stated) (Egestad et al., 1996). The radiolabelled DEHP was dilluted with unlabelled DEHP to give a specific activity of 2.7 and 6.1 μCi/mmol for the guinea pigs and mice, respectively. Urine was collected over 48 hours. Following extraction, individual metabolites were purified and separated using a combination of ion-exchange chromatography and reversed-phase HPLC. Analysis of intact conjugates, as well as nonconjugated metabolites, was performed by GC/MS. Enzymatic methods were used for further characterisation. The study confirmed glucuronidation as the major conjugation pathway for MEHP in the investigated species. The recovery of ^{14}C was 83-103% and 74-78% in guinea pigs and mice, respectively. In guinea pigs MEHP glucuronide were the dominating metabolite whereas in the mice it was an even distribution of the glucuronides of MEHP and its metabolites. In mice approximately 3% of the administered dose was found in the urine as ß-glucose conjugates. The ß-glucose conjugates were not observed in the guinea pigs.

Guinea pig

Male Hartely guinea pigs, rats, mice, and hamsters were given two doses of (carbonyl-^{14}C)DEHP by stomach tube at 24 hours intervals to compare the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982b). The study is presented in the section on oral administration to Fischer 344 rats.

Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. **Table 4.26** gives the distribution of radioactive metabolites in pools of urine from three animals, however, it should be noted that the doses were not the same between species. No components were detected in urine from hamsters or guinea pigs that were not also present in urine from rats and mice. In the hamsters the main metabolites were: di methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating metabolite. MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete

conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the species tested. **Table 4.27** gives the forms of excretion products in urine.

A single oral dose of (carbonyl-^{14}C)MEHP was given to male guinea pigs (Dunkin Hartely: number of animals not stated) and mice to compare the toxicokinetic behaviour of MEHP (Egestad et al., 1996). The study is presented in the section on oral administration to mice. The study confirmed glucuronidation as the major conjugation pathway for MEHP in the investigated species. The recovery of ^{14}C was 83-103% and 74-78% in guinea pigs and mice, respectively. In guinea pigs MEHP glucuronide were the dominating metabolite whereas in the mice it was an even distribution of the glucuronides of MEHP and its metabolites. In mice approximately 3% of the administered dose was found in the urine as ß-glucose conjugates. The ß-glucose conjugates were not observed in the guinea pigs.

Hamster

The elimination of DEHP was studied in rats and hamsters (Lake et al., 1984b). A single dose of (carbonyl-^{14}C) DEHP (> 99% pure) was administered to 5-week-old male Sprague-Dawley rats and male DSN strain Syrian hamsters at dose levels of 100 (5 rats, 3 hamsters) or 1,000 mg/kg bw (5 rats, 5 hamsters) in corn oil by gastric intubation. The study is presented in the section on oral administration to Sprague-Dawley rats. Urine and faeces were collected over a period of 96 hours and then the animals were sacrificed. Radioactivity was measured in urine, faeces, and total gut contents by liquid scintillation spectrometry. Faecal metabolites were extracted and chromatographed on thin-layer plates. In both species the bulk of the radioactivity was excreted within 24 hours. At the lower dose level, both species excreted more radioactivity in the urine (rat: 51%, hamster: 53%) than in the faeces (rat: 43%, hamster: 31%), whereas at the higher dose level, the major route of excretion was via the faeces (rat: 53%, hamster: 48%). In both species and at both dose levels, only negligible amounts of radioactivity were present at termination in either the liver, kidney, or total gut contents. Faecal radioactivity profiles were determined in 0-24 hour faeces samples. About 50% of the faecal radioactivity of rats at the higher dose level appeared to be the parent compound, the remainder comprised metabolites possibly including MEHP. In contrast, more than 95% of the faecal radioactivity of hamsters appeared to be the parent compound. Similar results were obtained with faecal extracts from rats and hamsters at the lower dose level.

Male guinea pigs, rats, mice, and Syrian golden hamsters were given two doses of (carbonyl-^{14}C)DEHP by stomach tube at 24 hours intervals to compare the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982b). The study is presented in the section on oral administration to Fischer 344 rats. Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. In **Table 4.26** the distribution of radioactive metabolites in pools of urine from the three animals and from other animals, however, it should be noted that the doses were not the same between species. No components were detected in urine from hamsters or guinea pigs that were not also present in urine from rats and mice. In the hamsters the main metabolites were: di methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating nmetabolite. MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the species tested. **Table 4.27** gives the forms of excretion products in urine.

Dog

A study to compare the toxicokinetic behaviour of DEHP between dog, rats and miniature pigs has been performed by Ikeda et al. (1980). The study is presented in the section on oral administration to Sprague-Dawley rats.

Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respectively; and after 4 days 37 and 53% (rats), 21 and 75% (dogs), and 79 and 26% (pigs), respectively. Elimination of radioactivity was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four radioactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolised DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 in all species and a small amount remained after 4 days. Bile samples from dogs, and to a lesser extent from pigs, accounted for a significant amount of administered ^{14}C dose. Less than 1% of the administered ^{14}C dose was secreted in the bile from bile duct cannulated rats.

Pigs

A study to compare the toxicokinetic behaviour of DEHP between miniature pigs, rat and dog has been performed by Ikeda et al. (1980). The study is presented in the section on oral administration to Sprague-Dawley rats.

Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respectively; and after 4 days 37 and 53% (rats), 21 and 75% (dogs), and 79 and 26% (pigs), respectively. Elimination of radioactivity was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four radioactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolised DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 in all species and a small amount remained after 4 days. Bile samples from dogs, and to a lesser extent from pigs, accounted for a significant amount of administered ^{14}C dose. Less than 1% of the administered ^{14}C dose was secreted in the bile from bile duct cannulated rats.

The distribution and retention of DEHP and DBP orally administered in feed to piglets has been studied (Jarosová et al., 1999). Six piglets (33-50 kg) of which 4 received DEHP (5 g per day and head (approximately 125 mg/kg bw/day) for 14 days and 2 served as controls were used. After 14 days, 2 treated and the 2 controls were sacrificed. The remaining two treated-piglets were then maintained on a DEHP-free diet, and one each sacrificed on day 14 and 28, respectively, post dosing. DEHP and MEHP were determined by HPLC analysis. Whole blood and urine samples were collected before sacrifice and on from treated animals on day 7 of treatment. The concentration of DEHP was measured in the whole (wet weight) and/or fat extracted tissues/organs of muscle, renal fat, subcutaneous fat, kidneys, lungs, brain, heart, and liver. MEHP was only determined in the whole liver, whole blood and urine samples.

Body and tissue/organ weights were not affected by administration of DEHP.

The highest levels of DEHP were in the subcutaneous (treated see control: approximately 19 see 0.42 mg/kg fat) and renal fat (approximately 25 see 0.37 mg/kg fat), muscle (approximately 25 see 2.4 mg/kg fat), heart (approximately 12 see < 0.2 mg/kg fat) and lungs (approximately 13 see 0.25 mg/kg fat). The amount of DEHP in kidney (approximately 2 see < 0.2 mg/kg fat) was low. MEHP, but not DEHP, level was increased in the liver, whole blood and urine: individuals values greatly varied but an increase up to, for example, of 20-1,000-fold in blood occurred.

DEHP was not increased in the brain. In the 14 day post dosing (recovery) animal, the level of DEHP was decreased by around 50% in subcutaneous and renal fat, muscle, heart and lungs. MEHP returned to control levels in liver, whole blood and urine. By 28 days, DEHP was reduced to control levels in all tissues/organs except in renal fat and the lungs. Although only one animal per recovery time interval was used, these data indicate that in piglets that DEHP is retained for a considerable time post dosing.

The authors further investigated the reason for the presence of DEHP in the organs (not blood) of the control animals. Analysis of the piglet feed showed that around 0.4 mg DEHP and 0.5 DBP per Kg commercial feed was present. Based on a 40 kg pig eating 2 kg of feed a day, the daily intake of DEHP is around 0.02 mg/kg bw. Comparing the daily intake (0.8 mg DEHP) with the residue of DEHP in whole muscle of control pigs (0.10 mg/kg), a biotransfer factor (BTF: concn. in meat (mg/kg)/daily intake of DEHP (mg/day)) of 0.125 d/kg is derived.

Broiler hens

The distribution and retention of DEHP and DBP orally administered in feed to broiler hens has been studied (Jarosová et al., 1999). Eighteen broiler hens (750 g) per treated and control group were used. DEHP (100 mg per day and head (approximately 135 mg/kg bw/day) was administered for 14 days. Six hens each per treated and control group were sacrificed at 14 days, and on post treatment days 14 and 28. DEHP and MEHP were determined by HPLC analysis. Liver and blood samples were obtained by heart puncture to determine DEHP and MEHP. Muscle (pooled samples of breast and thigh samples), skin (thoracic area), and mesenterial fat were analysed for DEHP. Post DEHP dosing samples of blood, muscle and skin were pooled from 6 individuals.

The highest levels of DEHP were in the mesenterial fat (treated see control: approximately 31 see 0.33 mg/kg fat), skin (approximately 26 see 3.8 mg/kg fat) and muscle (approximately 26 see 2.5 mg/kg fat). DEHP (approximately 6.3 see 0.47 mg/kg fat) and MEHP (approximately 0.15 see < 0.01 mg/kg whole tissue) were detected in the liver. In whole blood, levels of MEHP varied but indicated an increase of more than 7-fold. In the 14 day post dosing (recovery) animal, the level of DEHP decreased by more than 50% in muscle, skin, adipose tissue and liver. However, by comparison with the all the control groups and the treated group, DEHP is apparently retained in the muscle, skin and adipose tissue around 30%). MEHP levels in the liver and blood were reduced to control levels by post recovery day 14.

The authors further investigated the reason for the presence of DEHP in the organs (not blood) of the control animals. Analysis of the hens feed showed that around 1.0 mg DEHP and 2.0 DBP per Kg commercial feed was present.

4.1.2.1.2 Inhalation

There are few studies available concerning the inhalation route of exposure. Studies available for humans are not strictly toxicokinetic studies but case studies of patients and in the working environment. There is only one toxicokinetic study with rats exposed to a DEHP aerosol.

Humans

In three preterm infants, artificially ventilated with PVC respiratory tubes, the estimated inhalative exposure ranged between 1µg/h – 4,200 µg/h (Roth et al., 1988; see also Section 4.1.2.6.2). During the fourth week of life in two of the infants DEHP, but not MEHP,

could be demonstrated in urine samples. The authors assumed that these findings were causally related to the exposure to high doses of DEHP released from the PVC tubes. DEHP was detected in the lung tissue (0.23 μg/g wet weight) but not in the liver tissue of one infant who died two weeks after birth.

For a determination of occupational exposure to phthalic acid (PA) and DEHP at a plant manufacturing DEHP from PA and 2-EH, personal air samples were obtained over five shifts for the determination of exposure to PA and DEHP. Pre- and post-shift urine samples were collected, simultaneously with air sampling, for the determination of total phthalates (Liss et al., 1985). Urine samples were obtained from 48 workers in jobs likely to be exposed to DEHP and PA and from 47 workers in jobs unlikely to be exposed. The total phthalate concentration in urine was determined by GC. A number of urine samples (those with the highest total phthalate) were also assayed for DEHP and for specific metabolites by GC/MS. Of 50 personal samples obtained, six contained levels above the analytical limit of detection (10 μg/sample), and the time-weighted average concentrations ranged from 20 to 4 110 μg/m^3 (mean 71 μg/m^3). The most heavily exposed workers had the highest mean post-shift urine phthalate concentration (geometric mean 7.6 nmol/ml), and also the greatest mean increase (4.4 nmol/ml) in pre-shift to post-shift urine phthalate levels. Twofold increases over the shift in total urine phthalate concentration, and post-shift phthalate levels of greater than 10 nmol/ml were observed in 8 of 32 chemical operators, but in none of 52 other workers. MEHP was not deteced in the urine samples.

The uptake of DEHP, in 9 workers in a boot factory and 6 workers in a cable factory, was studied by determining the concentrations of four metabolites of DEHP in urine samples (Dirven et al., 1993a). When the workers started work on the first day in the working week, no occupational DEHP exposure had occurred during the last 48 hours. Urine samples were collected on the first and last day of the 5-day working week (boot factory) or the first and fourth day of the working week (cable factory), before work and after work. The concentration of the following metabolites of DEHP were determined with GC/MS: MEHP and metabolites V, VI and IX.

Personal air sampling (2 hours) was employed on the first and last day of the working week (boot factory) or on the first day of the working week (cable factory). Within each plant exposure levels were determined (by GC) for workers working on the mixing as well as for workers working on the extruding processes. In the boot factory, mean concentrations were 261 (100-1,214) μg/m^3 in the mixing area and 120 (48-278) μg/m^3 in the extruding area. In the cable factory, mean concentrations were 180 (9-809) μg/m^3 in the mixing area and 239 (10-1,266) μg/m^3 in the extruding area.

For workers in the boot factory, median concentrations of all four metabolites were comparable in the urine samples collected on day 1 and day 5 at the start and at the end of the work-day. On day 1 as well as on day 5, the concentrations of MEHP and metabolites V, VI and IX were significantly increased (1.2-2.3-fold) in urine samples collected at the end of the work-day compared to urine samples collected at the start of the work-day. For workers in the cable factory, median concentrations of all four metabolites were comparable in the urine samples collected on day 1 and day 4 at the start of the work-day but not in urine samples collected at the end of the work-day. On day 1 as well as on day 4, the concentrations of MEHP and metabolites V, VI and IX were significantly increased (1.2-4.5-fold) in urine samples collected at the end of the work-day compared to urine samples collected at the start of the work-day.

In another study by Dirven et al. (1993b), a method for biological monitoring of exposure to DEHP is described. The method in which the four main metabolites of DEHP (MEHP and

metabolites V, VI and IX) are determined in urine samples (GC/MS) was used to study DEHP metabolism in man. The quantitative distribution of the metabolites in urine were: MEHP, 26.2%; V, 21.8%; VI, 18.2%; IX, 33.8%. No details of exposure conditions are given, but the metabolites were detected in urine from workers occupationally exposed to DEHP by inhalation. Metabolites IX and VI were almost completely conjugated in all five persons (IX: 88-100% conjugated; VI: 80-95% conjugated) while 32-45% of metabolite V was present in conjugated form. For MEHP, a large interindividual variation was found with 77% and 100% present in free form in two persons but only 20, 30 and 38% in the other three persons. Interindividual variation in the metabolism of DEHP was examined in post-shift urine samples of 15 exposed men by comparing the amount of metabolite V (product of ω-oxidation) with the amount of metabolite VI and IX (products of (ω-1)-oxidation). No substantial interindividual differences were found. The absolute concentration of metabolites VI and IX was 1.7 times as high as the concentration of metabolite V indicating that (ω-1)-oxidation is favoured in humans.

Rats

In a study performed by General Motors (1982ab), adult male Sprague-Dawley rats (200 g) were exposed, either once or repeatedly, by inhalation to a DEHP aerosol (98% pure). In the single exposure study, six rats were exposed to 129 ± 26 mg/m^3 of (carboxyl-^{14}C) DEHP for 6 hours (head-only chamber; radiochemical purity of the labelled compound 95%). Accurate particle size determination was not technically possibly to conduct; however, the particle size was estimated to be 0.4 - 0.5 μm. Three animals were sacrificed immediately after exposure and the remaining three after the follow-up time. The follow-up time for collection of urine and faeces samples was 72 hours before the animals were sacrificed. Samples were collected at 12- and 24-hour intervals for urine and faeces, respectively, and assayed for radioactivity. Blood samples (0.1 ml) were taken from the jugular vein 1, 3, 6, hr during exposure and 1, 3, 6, 12, 18, 24 and 36 hours post-exposure. Tissues (lung, liver, kidney, fat, adrenal, heart, spleen, thymus, testes, and brain) were removed post mortem and assayed for radioactivity. Radioactivity was determined by liquid scintillation spectrometry. Metabolites were determined by HPLC in urine samples collected between 0-12, 12-24, 24-36 hours. Data were calculated as μmole equivalents DEHP.

During single dose inhalation exposure (^{14}C) DEHP was absorbed rapidly as indicated by the amount of radioactivity in the blood. Following exposure, the decrease in radioactivity in blood was log non-linear. Immediately after exposure, approximately 1 mg (based μmole equivalents DEHP) or 75% of the body burden was recovered in the carcass and skin. 10% was recovered in the lung and approximately 2% in all the other tissues excluding the brian where no radioactivity was detected. Radioactivity associated with the lung probably represents that fraction of aerosol particles deposited in the larger airways. This fraction could be absorbed through the gastrointestinial tract following clearance from the pulmonary spaces by mucociliary ladder mechanism. It is probable that a large portion of DEHP is ingested. After 72-hours, approximately 1.5 mg (3.94 μmole equivalents DEHP) was recovered mainly in the urine and faeces. Combined, urine (52%) and faeces (40%) accounted for greater then 90% of total recovered radioactivity. Around 6% of the original body burden radioactivity was determined in tissues (low amount of radioactivity in the lung and liver, and trace amount in kidney), carcass, and skin. Baesd on this information the retention of DEHP can be derived by comparison with the calculated inhaled dose. Assuming a minute volume of 0.2 l/min and 100% inhaltion of the aerosol the inhaled dose is around 36 mg/kg bw/day or 7.2 mg/rat/day for a 6 hour per day inhalation exposure. Retention is thus around 21% (1.5/7.2) for DEHP with an estimated particle size of 0.4 - 0.5 μm.

The rate of excretion of radioactivity in the faeces was approximately first order kinetics during 72-hour. The elimination half-life was approximately 22 hours and the elimination rate constant (Ke) 0.032 hr^{-1}. In urine, excretion was biphasic. The initial rapid phase was approximately 10 hours (Ke = 0.069 hr^{-1}). And was sustianed for 30 hours. The slower phase half-life was 22 hours.

At least three peaks were identified by HPLC in urine. Phthalic acid (3-5% total radioactivity) was identified. The reamining radioactivity was confined to two other peaks, however, the metabolites were not identified: DEHP was not detected in the urine.

Under the experimental conditions used in this study, around 1.5% of a nominal aerosol concentration of 100 mg/m^3 was absorbed by rat following a 6 hour exposure period. Both pulmonary and gastrointestinial tract absorption are expected to contribute to the total body burden level of detected ^{14}C-DEHP derived radioactivity.

For comparison, General Motors (1982ab) also studied the disposition in male Sprague-Dawley rats (200 g)(number not stated) after a single peroral (gavage) dose of (carboxyl-^{14}C)DEHP (25 μmoles/kg bw (≈10 mg/kg bw): 25 μmoles/ml, 9.7 μCi/ml) administered in corn oil with the results of the single exposure inhalation study (see above). The dose was selected to approximate DEHP absorbed during inhalation (≈ 2 mg/animal). Faeces were collected over 24 hours interval and urine over 12 hours inteval. The animals were killed after 72 hours Tissues (lung, liver, kidney, fat, adrenal, heart, spleen, thymus, testes, and brain) were removed post mortem and assayed for radioactivity. Radioactivity was determined by liquid scintillation spectrometry. A more rapid excretion of ^{14}C- in the urine and increased body burden clearance (half-life ≈ 12 hours) was observed following oral administration compared with inhalation exposure. The percentage cumulative recovery of radioactivity in urine (54%) and faeces (43%) after 72 hours was not significantly different as compared with inhalation exposure (52 and 40%, respectively, in urine and faeces). Within 72 hours the recovery was 3.94 and 3.82 μmole equivalents DEHP (approximately 1.5 mg) following single oral and inhalation administration, respectively. To assess the relevance of this comparison route-specfic metabolism, biliary exretion and reabsorption, and the contribution of GI-tract absorption during and following inhalation exposure should be considered compared with unabsorbed orally administered DEHP. Urinary excretion following single oral exposure was also biphasic with half-lifes of about 10 and 22 hours, respectively. The excretion in urine and decline in body burden were more rapid following oral administration, especially initially.

In the repeated exposure study General Motors (1982ab), 16 rats were pretreated with 100 mg/m^3 unlabelled DEHP for 2 weeks (6 hours per day, 5 days per week in an exposure chamber, mass median aerodynamic diameter of aerosol particles: 0.6 μm) except that the last exposure was to (carboxyl-^{14}C)DEHP (head-only chamber).

Following repeated inhalation exposure, around 90% of the radioactivity was excreted in the urine (50%) and faeces (40%), and 8% recovered in the carcass and skin. Excretion of radioactivity in urine was apparently first order with a half-life of about 25 hours. Urinary excretion seems to be initially slower compared with single inhalation exposure (see above) but was parallell to the curve from single exposure after less than 24 hours.

The study indicates that following repeated inhalation exposure long-term retention does not occur and that the excretion profile is not modified by prolonged inhalation exposure (2-weeks) compared with single exposure. Hence, disposition characteristics following repeated exposure were similar to single dose exposure.

4.1.2.1.3 Dermal

Humans

Percutaneous absorption of a signal application of (^{14}C) DEHP (> 98% radiochemical purity) was determined in a human volunteer study (Wester et al., 1998). The study is poorly reported as identification of DEHP is ambiguous as non standard and incorrect terms are used throughout the report. Six "normal" volunteer outpatients were topically dosed with (^{14}C)DEHP (18.5 μg/cm^2) dissolved in 50 μl ethanol spread over10 cm^2 on the ventral forearm. Ethanol was allowed to evaporate and the site was not occluded. Subjects were instructed not to touch or wash the study area for 24 hours; however, they were allowed to wear clothing of their choice over the dosing area. After 24 hours, the dosing site was washed and rinsed to determine the surface fraction of non-absorbed DEHP. Only urine was collected per individual from application to 7 days to determine systemic available (^{14}C). 7 days after the application the skin dosing site was cellophane tape-stripped 10 times to determine the residual (reservoir) amount (^{14}C)-label. The percent of (^{14}C)-label recovered was 4.5% (skin surface wash; at 24 h), 1.1% (cumulative urine excretion; for 7 days), and 0.15% (tape strip recovery; at 7 days). To correct the dermal absorption for the fraction of dose excreted by other routes (e.g. faeces) or retained in the body, Rhesus monkeys (used as human surrogate) were given an intravenous dose of DEHP. 7 days after *i.v.* administration of (^{14}C)DEHP (1.1 μCi in 0.5 ml propylene glycol) to 4 female Rhesus monkeys the cumulative recovery of (^{14}C)-label was 61% indicating that either bilary excretion and/or retention had occurred. Following this correction the material mass balance recovery considerable less than 100%. The authors suggest this to be due to lose by evaporation and/or on clothing during 24 hour exposure period following application. The duration for which DEHP was in contact with the skin during the 24-hour period is not known precluding an accurate quantitative determination of dermal uptake. Hence, this study is not considered for risk characterisation.

Rats

Dermal absorption distribution, and excretion were studied in rats by Elsisi et al. (1989). Hair from a skin area (1.3 cm in diameter) on the backs of male F344 rats (number not stated) was clipped, (^{14}C)DEHP (> 96% radiochemically pure, uniformly labelled on the ring) was applied ast a single dose of 30-40 mg/kg bw (5-8 mg/cm^2) in ethanol and after evaporation the area of application was covered with a perforated cap (nonocculded). Rats were kept in metabolic cages and urine and faeces were collected every 24 hours for 7 days. On the 7th day the rats were sacrificed and samples from various organs and tissues (brain, spinal cord, lung, liver, spleen, intestine, kidney, testis, fat, muscle, and skin) were collected. The skin area of application was also removed and analysed. The radioactivity was determined using liquid scintillation spectrometry. Cumulative excretion in the urine and faeces was around 4.5%. The amount of radiolabel remaining in the body 7 days after dosing was less than 2% of the applied dose. Retention in the different organs and tissues examined was low ≥ 0.3%: muscles showed the highest amount 1.17%. Most of the unabsorbed dose (86%) remained at the skin area of application after 7 days. Dermal absorption (considered as the cumulative amount detected in excreta and tissues, excluding the dosed skin (a lower bound)) is calculated to be 6.5%.

The dermal absorption of (^{14}C) DEHP was evaluated in male F344 rats (Melnick et al., 1987). A single dose of 30 mg/kg bw (6 mg assuming 200 g/rat: 4.5 mg/cm^2) of (^{14}C) DEHP (purity and position of label not stated), dissolved in ethanol, was applied to a circular area of 1.3 cm diameter (1.326 cm^2) in the middle of the back of three rats. After the ethanol had evaporated, a perforated plastic cap was glued on the skin over the site of application. Urine and faeces were

collected every 24 hours for 5 days and radioactivity was determined by liquid scintillation spectrometry. Five days after dosing, recovery of ^{14}C in the urine and faeces was around 5% with 3%, respectively. Body organs and the skin in the area of DEHP application site were also collected and analysed. The amount of radiolabel retained in the body 5 days after dosing was less than 2% of the applied dose. The highest amount was recovered in the muscle (1.2%). About 95% of the applied dose was recovered from the application site and the plastic caps which were used to cover the application site. These results indicate that DEHP is not well absorbed through the skin of rats. Dermal absorption (considered as the cumulative amount detcted in excreta and tissues, excluding the dosed skin (a lower bound)) is calculated to be 9%.

The absorption of (^{14}C)DEHP contained in a PVC plastic film (40% w/w (25.5mg/cm^2): 0.5 mm thick) in male Fischer 344 rats was studied (Deisinger et al., 1998). Sheets of PVC film (15 cm^2) were applied to the shaved backs of eight rats in two separate experiments (4 rats/experiment). In a short term study ("study II"), the PVC was removed after 24 hours and the animals killed. In a longer term study ("study I"), after removal of the PVC film at 24 hours the animals were rewrapped with aluminium foil and bandage at the exposure site and sacrificed after a further 6 days. In both studies, (^{14}C)-label was determined in the following: urine and faeces up to sacrifice; cage washes; washed and rinsed residue from the exposure site before sacrifice; entire clipped area including the dermal exposure site was exercised after washing and rinsing; the remainder of the body (carcass). The PVC films were also analysed for remaining (^{14}C) DEHP after the 24-hour exposure period. In "study I" (longer term study), the aluminium and bandage used from 24 hours to 7 days were analysed. The migration of (^{14}C) DEHP from the film was 261 and 505.6 µg during 24 hours (0.725 and 1.4 µg/cm^2/hour). Based on the materials mass balance information on the combined amount of (^{14}C)DEHP for urine, faeces, cage washes and carcass, and residual amount in the skin at the application site, the authors calculate that the percutaneous absorption rate (J) is around 0.24 µg/cm^2/hr in both studies.

Guinea pig

Dermal absorption of a single dose radio-labelled DEHP was determined in 5 female hairless guinea pigs [Crl:LAF/HA(hr/hr)BR, 20-30 weeks old] (Ng et al., 1992). (Carbonyl-^{14}C)DEHP (53 µg, 13.2 µg/cm^2, > 98% pure) was dissolved in 50 µl acetone and applied topically on 4 cm^2 of the washed upper dorsal area. The exposed area was then covered by a protective nonocclusive pad to prevent ingestion of the compound. Animals were kept individually in glass metabolism cages. 24h after dosing the pad was removed and the dosing site cleansed with soap and water to remove unabsorbed material. Urine and faeces were collected at 6 and 12 hours on the first day and then at daily intervals for 7 days post-treatment for radioassay. Seven days post-administration the site of application was stripped with tape 10 times. Radioactivity content on the tape was determined by liquid scintillation spectrometry. To correct the dermal absorption for incomplete excretion (e.g. body retention), the excretion rate of an intramuscular dose, in 5 animal, was compared with the excretion rate of a topical dose.

Three percent (7% after correction) of the dermally administered dose was absorbed *in vivo* and excreted in 24 hours. Around 60% of the topical dose was not excreted in the faeces and urine after 7 days. The percent of (^{14}C)-label recovered was 31% from skin surface wash (performed at 24 hours), 53% (21% before corrected) cumulative urine and faeces excretion up to 7 days, and 11.3% was tape strip recovery (performed at 7 days). 13% and 5% were recovered in the protective skin pad and body tissues (liver, fat, muscle, skin), respectively. The total recovery was 95% (76% before corrected). To determine the amount of DEHP that might have been have volatilized during the penetration process, DEHP was applied on a piece of skin that was kept in a petri dish at 33ºC for 7 days. Analysis of the radioactivity content revealed that 10% of the

dosed material was lost. Thus volatilisation can only in part account for the loss of DEHP from the application site. Dermal absorption (considered as the cumulative amount detcted in excreta and tissues, excluding the dosed skin (a lower bound)) is calculated, without consideration of correction, to be 26%.

The skin reservoir and bioavailability of dermally administered (^{14}C) DEHP in hairless guinea pigs was determined (Chu et al., 1996). Different amounts of (^{14}C) DEHP were applied to the washed dorsal region of 4 female Hartely hairless guinea pigs in four different experiments for different times and dosages: 119 μg/cm^2 for 24 hours; 107 μg/cm^2 for 48 hours; 442 μg/cm^2 for 7 days; and, 529 μg/cm^2 for 14 days. Radioactivity was measured in skin sections by autoradiography or liquid scintillation to determine the amount of radioactivity. The authors conclude that the results indicate that the amount of DEHP remaining in the skin after washing will eventually enter the systemic circulation and should be considered as part of the total dose absorbed, and that the hair follicle may play a role in percutaneous penetration. Dermal absorption (considered as the cumulative amount detcted in excreta and tissues, including skin dose) is calculated to be 9.7-18.9% from the four different experiments.

In vitro

Absorption, permeability constant and percutaneous absorption rate of (^{14}C)DEHP was determined in the epidermis of nonviable human skin (autopsy sample) and rats (AL/pk, Wistar-derived) with a glass diffusion cell (Scott et al., 1987). 0.5 ml (^{14}C)-labelled undiluted DEHP was applied to the skin preparations. Apparently the skin discs were 7 cm^2, therefore, 70 mg/cm^2 (^{14}C) DEHP was applied. 50% v/v aqueous ethanol was used as the receptor fluid and 50 μl samples collected at regular intervals. Diffusion cells were maintained at 30ºC. The results are presented in **Table 4.20**.

Table 4.20 Absorption rate data of DEHP. Adapted form Errata to Scot et al. (1987)

	Human*	Rat*
Permeability constant (K_p: 10^{-5} cm/hr)	0.57	2.28
Percutaneous permeability rate (J: μg/cm ^{22}/hr)	5.59	22.37
Lag time (hr)	3.1	3.9

* Epidermis, 50% aqueous ethanol

Absorption, permeability constant and percutaneous absorption rate of (^{14}C) DEHP was determined in the stratum corneum of human skin (autopsy sample) and full thickness rat skin Fischer-334 with a Franz-types diffusion cell (Barber et al., 1992). 0.3 ml (^{14}C)-labelled undiluted DEHP was applied to 1.02 or 0.636 cm^2 of skin (288-576 mg/cm^2). The receptor solution was based on Dulbecco's phosphate-buffered (pH 7.1) isotonic saline. Diffusion cells were maintained at 37 or 30ºC. The results are presented in **Table 4.21**.

Table 4.21 Absorption rate data of DEHP. Information are adapted

	Human*	Rat**
Permeability constant (K_p: 10^{-5} cm/hr)	0.0105	0.0431
Percutaneous permeability rate (J: μg/cm^{22}/hr)	0.1	0.42

* Stratum corneum.
** Full thickness skin. Buffer

The permeability constant of (^{14}C) DEHP in the epidermis and dermis of male Spague-Dawley rats was compared using buffer or aqueous ethanol with a diffusion cell (Pelling et al., 1998). Absorption was determined using both phosphate buffer saline and 50% aqueous ethanol as receptor medium. (^{14}C)DEHP was applied in 50 µl acetone (78.6 µg; 78.6 µg/cm^2 assuming application to 1 cm^2). Diffusion cells were maintained at 31.5ºC. The results are presented in **Table 4.22**. The authors comment that the rate and extent of DEHP absorption through the epidermis was greatly increased (40- and 80-fold, respectively) using 50% aqueous ethanol as receptor fluid compared with buffer. Also in comparison with Scott et al. (1987) it is noted that the K_p for the aqueous ethanol system is much higher (16-fold) in this study.

Table 4.22 Absorption rate data of DEHP. Information are adapted

	Rat	
	Buffer	**50% aq. Ethanol**
Permeability constant (K_p: 10^{-5} cm/hr)		
Epidermis	1.3	94.6
Dermis	4.76	9.83
Percutaneous permeability rate (J: µg/cm ^{2}/hr)*		
Epidermis	0.02	0.786

* Calculated based on data detailed in the study. The area of the applied (^{14}C)DEHP was assumed to be 1 cm^2

The absorption of (^{14}C) DEHP was determined in full thickness viable and nonviable guinea pig skin with a diffusion cell (Ng et al., 1992). The receptor solution was based on Hepes-buffered Hanks' balanced salt solution, containing gentamicin (50 mg/l) and 4% bovine serum albumin. (^{14}C)DEHP was applied in 10 µl acetone (53.2, 228 and 468 µg/cm^2). Diffusion cells were maintained at 37ºC. The amount of (^{14}C) recovered in receptor fluid, on skin disc and in skin wash after 24 hours was determined. *In vitro* experiments were also carried out using perfusate containing an esterase inhibitor, phenylmethylsulfonyl fluoride (174 mg/l). Metabolites were identified by GC/MS.

Total recovery was between 78-90%. The results are presented in **Table 4.23**. The authors comment that for a dose of 53.2 µg/cm^2 the *in vitro* absorption of 47% (receptor fluid + skin disc) after 24 hours is comparable with the corrected *in vivo* cumulative excretion (urine + faeces) of 53% after 7 days (see Ng et al., 1987 1992 under: toxicokinetics; dermal; guinea pig). To determine the amount of DEHP that may have volatised during the penetration process, DEHP was applied on a piece of skin that was kept in a petri dish at 33ºC for 7 days. Analysis of the radioactivity content revealed the 10% of the dosed material was lost. Thus volatilisation is apparently low and can only in part account for the lose of DEHP from the application site. DEHP was metabolised to a monoester, MEHP. In the presence of the esterase inhibitor, the dose that permeated into the receptor fluid decreased from 3.36% in the control to 2.67% in 24 hours. The proportion of MEHP was also reduced from 2.36 to 1.23% in the inhibitor treated group.

Table 4.23 Percent radiolabel recovered for DEHP at 24 hr post application. Information are adapted

	Dose (μg/cm²)			
	53.2	**53.2***	**228**	**468**
Receptor fluid	6.1	5.0	2.4	2.5
Skin disc	41	40	37	36
Receptor fluid + skin disc	47	45	40	39
Skin wash	38	41	50	40
Total recovery	85	86	90	78
Estimate Per Ab rate (J: μg/cm²/h)#	**0.13**	**0.11**	**0.23**	**0.49**

* Nonviable skin.

\# Calculated based on % in receptor fluid and 24 hours. Information on the rate of excretion is not detailed in the study other than for at 24 hours.

The absorption of (^{14}C) DEHP was determined in the perfused porcine skin flap (Wester et al., 1998). Isolated skin flaps were perfused in a nonrecirculating chamber. 10 cm^2 was dosed with (^{14}C) DEHP (18.5 μg/cm^2). After 8 hours, 0.14% was recovered in the perfusate, 14.5% was recovered in skin strips (taped 12 times), 3.8% in the remaining skin and 71% in skin surface wash, a total material balance recovery of 94%. Based on the combined amount in the perfusate and skin strips (14.6%) the percutaneuos absorption rate is 0.34 μg/cm^2/hour.

4.1.2.1.4 Other routes

Exposure of humans to DEHP through medical treatment practices such as dialysis, respiration therapy, blood transfusions, or parenteral nutrition where the source of DEHP is the plastic materials used in the medical treatment devices or storage bags may also occur. The results detailing the amounts of DEHP/MEHP that humans are internally exposed to are presented in Section 4.1.1.2.5. More detailed information on selected studies is presented in this section. In addition, levels of DEHP found in biological media but with unknown exposure pathways are detailed.

Humans

Adults

DEHP was found to be extracted from polyvinyl chloride plastic blood bags by both human and dog blood stored at 4°C at a rate of 0.25 mg/100 ml per day (Jaeger and Rubin, 1972). Seven of 12 lung-tissue samples taken at autopsy from patients who had received transfusions of stored blood contained detectable amounts of DEHP. One patient, after open-heart surgery, excreted both free phthalic acid and hydrolysable phthalic-acid-containing metabolites of DEHP in excess of the amount calculated to have been received in the transfused blood. The authors suggest that the excess came from the tubing of the by-pass system.

Six adult leukemia patients receiving platelet concentrates containing 18-38 mg/dl of DEHP (platelets stored for up to 26 hours in vinyl plastic bags) showed peak plasma levels of DEHP levels from 0.34 to 0.83 mg/dl at termination of the transfusion (Rubin and Schiffer, 1976). The DEHP concentration in the blood declined with a mean half-life of 28 minutes. Urinary excretion investigated in two patients showed 60 and 90% excretion of the total administered amount following 24 hours post transfusion.

Two cancer patients received larger doses of DEHP (case I: 95 mg in 4 hours., case II: 174 mg in 1.5 hour., respectively) via platelet concentrate infusions (Peck and Albro, 1982). More than 50% of the infused dose appeared as DEHP derivatives in the urine within 6 hours. Approximately 80% of the urinary metabolites were conjugated to glucuronide. The metabolites from case II were detected in urine by GC/MS. The metabolites in urine were identified as MEHP (6.9%), and metabolites II (1.2%), IV (1.5%), V (4.7%), VI (7.7%), VII (8.3%), VIII (6.1%), IX (23.4%) and X (trace) (cumulative % of dose 24 hours after infusion), numbered according to Albro et al. (1983).

DEHP was assessed in 11 patients undergoing maintenance haemodialysis for the treatment of renal failure (Pollack et al., 1985b). On the average, an estimated 105 mg (range 24-360 mg) of DEHP was extracted from the dialyzer during a single dialysis session. Time-averaged circulating concentrations of DEHP and MEHP during dialysis were similar (1.91 and 1.33 μg/ml, respectively). Blood concentrations of phthalic acid (5.22 μg/ml) were higher than those of the esters. The length of the time patients had been receiving regular dialysis treatment was not determinant of circulating concentrations of DEHP and MEHP, but of the circulating concentrations of phthalic acid. The presence of significant amounts of MEHP and phthalic acid in circulation following exposure to DEHP via hemodialysis means that the potential contribution of the biologically active de-esterified products to the long term toxicological effects of DEHP must be recognised.

The concentration of DEHP was measured in serum of four chronic haemodialysis patients (2 males and 2 females, 1-8 years hemodialysis) pre- and post-dialysis (Malik et al., 1993). Pre-dialysis concentrations ranged from 0.01 to 0.25 μg/ml. There was a strong positive correlation between the post-dialysis concentration (0.40 to 1.01 μg/ml) and the number of years patients had been on dialysis.

Phthalate monoester metabolites have been determined in urine samples from a reference population of 289 adult humans in USA (Blount et al., 2000). The urinary samples were collected during 1988-1994 from adults aged 20-60 years (37.4 ± 10.6 years; mean ± S.D.). The gender distribution (56% females) was similar across age groups. Racial distribution was weighted toward minority groups (Caucasian 39%. African American 30%, Mexican American 23%, and other 8%). Randomly selected urine samples from 289 subjects were analysed for phthalate monoesters. Samples were treated with a β-glucuronidase, lacking lipase and sulphatase activity, to release phthalate ester from its conjugated form. Chromatographic separation by HPLC was followed with tandem mass spectrometry using atmospheric pressure chemical ionisation. Although the exposure source and route are not detailed in this study, it may be assumed that exposure occurs from the consumer setting and via the environment.

The amount of the DEHP monoester (MEHP) detected was 15.2 μg/g creatine (95th percentile). Other phthalate monoesters were detected (95th percentile): monoethyl phthalate (2,610 μg/g creatine); monobenzyl phthalate (91.9 μg/g creatine); monobutyl phthalate (162 μg/g creatine); monocyclohexyl phthalate (1.0 μg/g creatine); monoisonyl phthalate (6.8 μg/g creatine), and; monooctyl phthalate (2.1 μg/g creatine). Women of reproductive age (20-40 years) were found to have significantly higher levels of monobutyl phthalate than other age/gender groups.

Due to the small population size, lack of information on the human exposure profile and, in general, limited toxicokinetic data in humans, these data are not used for risk characterisation.

Phthalates levels have been determined in the serum of girls with premature breast development (thelarche) (Colón et al., 2000). Based on the data accumulated from 1984 to 1993, the estimated annual average incidence rate of premature thelarche in Puerto Rican girls 6-24 months of age is

8 ceases per 1,000 live female births. Case subjects diagnosed with thelarche were females from 6 months to 8 years of age (mean age 31 months; median 20 months), all samples (41) were taken from thelarche patients from January 1994 to April 1998. Control subjects were female from 6 months to 10 years of age (mean age 70 months; median 46). Controls (35) were undergoing general paediatric care and did not show evidence of premature sexual development or endocrine diseases. Blood was collected, serum isolated and extracted for organic compounds. Recovery was > 80% for all compounds. The extracts were analysed by GC/MS. Care was taken through out the sampling and analysis to determine contamination from trace amounts of phthalates.

DEHP was detected in 25/41 thelarche subjects (average: 450 ppb) and 5/35 control subjects (average: 70 ppb): this difference was statistically significant. MEHP was only detected in 3/41 thelarche subjects (average: 3 ppb) but not control subjects. Dibutyl phthalate (average 42 ppb: 13/41), diethyl phthalate (average 3 ppb: 5/41) and benzyl butyl phthalate (average 4 ppb: 2/41) were detected in thelarche subjects but not controls. The authors do not propose that higher amounts of serum DEHP cause thelarche, but propose that further research should be conducted to determine the role of phthalates

The relevance of this study for risk characterisation is at present unclear.

New-born infants

Four newborn infants receiving exchange transfusions were studied (Sjöberg et al., 1985a). Three of the infants were pre-term, the length of gestations were 31, 25 and 35 weeks, and one of the infant was full-term (length of gestation was 37 weeks). In three of the infants repeated exchange transfusions were made. During a single exchange transfusion the amounts of DEHP and MEHP infused ranged from 0.8-3.3 and 0.05-0.2 mg/kg bw, respectively. There were indications that about 30% of the infused DEHP originated from parts of the transfusion set other than the blood bag. Approximately 30% of the infused amount of DEHP was withdrawn during the course of each transfusion. Immediately after the transfusions the plasma levels of DEHP ranged between 5.8 and 19.6 μg/ml and subsequently they declined rapidly. This decline, probably reflecting distribution of DEHP within the body, was followed by a slower elimination phase. The half-life of the elimination phase was 10 hours. The maximal plasma levels of MEHP were about 5 μg/ml. In one pre-term infant the elimination of MEHP was slower than its formation whereas in one full-term newborn the formation appeared to be rate-limiting for the elimination.

Developed methods for the analysis of conjugated and unconjugated metabolites of DEHP were applied to the analysis of urine from a newborn infant after an exchange transfusion on day 3 performed due to hyperbilirubinemia (Egestad et al., 1996). During the transfusion 170 ml blood per kg bw was exchanged. Urine was collected for 8 hours and metabolites were excreted in amounts which could be analyzed by fast atom bombardment mass spectrometry. The fraction of metabolites conjugated with glucuronides was dominated by several metabolites from DEHP but no conjugated MEHP was found. According to the authors, and in comparison with data from earlier studies in adults there is a somewhat higher capacity for oxidation of MEHP in newborns.

The exposure of six newborn infants to DEHP and its primary metabolite MEHP was studied during exchange transfusion by measuring their contents in the infused blood (Sjöberg et al., 1985b). Five of the infants were born at term, and one was pre-term born after 36 weeks of gestation. Two of the infants received two transfusions with an interval between the first and the second transfusion of 16 and 23 hours, respsctively. Plasma concentrations of DEHP and MEHP in the blood withdrawn from the infants during the transfusion were also determined. The

amounts of DEHP and MEHP infused varied from 1.7 to 4.2 and 0.2 to 0.7 mg/kg bw, respectively. Immediately after the transfusions, the plasma levels of DEHP in the individual infants varied between 3.4 and 11.1 μg/ml. MEHP in the corresponding samples varied between 2.4 and 15.1 μg/ml. Judging from the plasma concentrations of DEHP and MEHP during and after transfusion, there was no gradual accumulation of these substances during the course of transfusions. In the two infants who underwent a second transfusion, significant levels of phthalates were found in plasma at 16 and 23 hours, respectively, after the first transfusion. Plasma concentrations of DEHP in these infants declined at a faster rate than those of MEHP, thus pointing to the importance of examining the pharmacokinetics of this poptentially toxic metabolite.

Non-human primates

The disposition of DEHP was studied in marmosets (Rhodes et al., 1983). Groups of three male marmosets received a single dose of (^{14}C-ring labelled) DEHP (radiochemical purity 97.5%) by the intravenous (100 mg/kg bw), intraperitoneal (1,000 mg/kg bw) and oral (100 and 2,000 mg/kg bw) routes. Urine and faeces were collected for seven days and the radioactive content determined. Following intravenous administration approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion for 7 days) indicating a 2 to 1 ratio between the urinary and biliary (faecal) routes of excretion in the marmoset. Around 28% of the dose remained in the lungs 7 days after administration of ^{14}C-DEHP, with minimal levels in other tissues. A much smaller proportion of the dose was excreted following intraperitoneal administration (10% in the urine and 4% in the faeces) in a similar 2 to1 ratio. Around 85% of the dose remained as unabsorbed DEHP in the peritoneal cavity with minimal amounts in the tissues (0.6%). The results for oral administration are presented above.

The Urinary excretion and metabolites of DEHP were studied in two African Green monkeys following a bolus infusion with ^{14}C-DEHP (carbonyl-^{14}C-DEHP-enriched plasma containing 96% DEHP and 4% MEHP) (Albro et al., 1981; Peck and Albro, 1982). To closely simulate the manner in which man is exposed to DEHP when receiving blood products, a ^{14}C-DEHP impregnated PVC plastic strip was immersed in 20-ml aliquot of plasma and stored for up to 5 months at 4°C. Serial plasma, urine and stool samples were obtained and measured. Urine metabolites were isolated and identified by GC/MS. Plasma C^{14} concentration rapidly decreased: less than 5% by 30 minutes post infusion. C^{14}-MEHP increased rapidly followed by ^{14}C-MEHP oxidation products. The levels of MEHP was greater than the oxidation products. MEHP is apparently quite stable since ^{14}C-MEHP in plasma increased to a plateau within 7 minutes and then then remained at a constant level for more than 30 minutes even though the total ^{14}C-plasma levels decreaseds in the plasma. The cumulative ^{14}C excretion in urine of three monkeys was 50% and greater than 70% by 4 and 24 hours, respectively. Urine samples from two monkeys were collected for a 5-hour post-infusion period. Approximately 80% of the urinary metabolites were excreted in the form of glucuronide conjugates (see **Table 4.27**). According to the authors the glucuronide frequency is analogous to what has been reported for human leukemia patients but in clear contrast to rats where rat urinary metabolites are excreted unconjugated. Seven metabolites (MEHP, V, VI, VII, VIII, IX, X, XI) were identified (GC/MS) with MEHP (29%) and metabolite IX (38%) being the major metabolites. The remaining metabolites amounted to 7% or less.

The effects of DEHP on hepatic function and histology were evaluated in the rhesus monkey undergoing chronic transfusion (Jacobson et al., 1977, see also Section 4.1.2.7.1). These studies demonstrates that intravenous administration of solubilized DEHP results in detectable concentrations of DEHP in biopsy material for up to 14 months following transfusion. The initial

liver biopsies (100-400 mg) of all the exposed animals contained significant amount of DEHP. Plasma or PRP (platelet-rich-plasma) stored at 22°C yielded higher levels of DEHP in the livers of the transfused animals than plasma or PRP stored at 4°C. The 5 month follow up samples contained concentrations similar to that initially measured. Since the post-transfusion samples were in the lower range of sensitivity one of the animals were killed 14 month post-transfusion and the level of DEHP in different organs was analysed. Significant levels were found in the liver (0.7%), testis (0.4%), heart (0.8%) and fat (2.0%). The residual organ level excluding fat was less than 1% of the dose administered. One animal had tuberculosis and was killed 5 month post-transfusion. In this animal significant levels were found in the spleen (10%), lung (15%), fat (20%) and liver (1.7%). Based on organ weight (excluding fat) and DEHP content, the residual DEHP was 5 percent of the dose administered, according to the authors.
To correct the dermal absorption for the fraction of dose excreted by other routes (e.g. faeces) or retained in the body, Rhesus monkeys (used as human surrogate) were given an intravenous dose of DEHP (Wester et al, 1998) (see section on Dermal exposure above). 7 days after *i.v.* administration of (^{14}C)DEHP (1.1 μCi in 0.5 ml propylene glycol) to 4 female Rhesus monkeys the cumulative recovery of (^{14}C)-label in urine was 61% (1.67:1 ratio) indicating that either bilary excretion and/or retention had occurred.

Rats

The disposition kinetics of DEHP was studied in male Sprague-Dawley rats following single or multiple administration of DEHP by various routes (intra-arterial: 100 mg/kg bw; intraperitoneal: 4,000 mg/kg bw; peroral: 2,000 mg/kg bw) (Pollack et al., 1985a, the study is described in section oral, rats).

The disposition of DEHP and four of its metabolites was studied in Sprague-Dawley rats (40-days-old, 8 in each group) given single infusions of a DEHP emulsion in doses of 5, 50 or 500 mg DEHP/kg bw (Sjöberg et al., 1985d). Plasma concentrations of DEHP, MEHP and metabolites V, VI and IX were followed for 24 hours after the start of infusion. The kinetics of the primary metabolite MEHP was studied separately. The concentrations of DEHP in plasma were at all times higher than those of MEHP which were much higher than the concentrations of the other metabolites investigated. In animals given 500 mg/kg bw the area under the plasma concentration-time curves (AUCs) of the other investigated metabolites were at most 15% of that of MEHP. Parallell decreases in the plasma concentrations of DEHP, MEHP and metabolites V, VI and IX indicated that the elimination of DEHP was the rate limiting step in the disposition of the metabolites. This was partly supported by the observation that the clearance of MEHP was higher than that of DEHP. Nonlinear increases in the AUCs of DEHP and MEHP indicated saturation in the formation as well as the elimination of MEHP.

Transplacental transfer of DEHP has been observed following intraperitoneal administration of (carboxy-^{14}C) DEHP on gestational day 5 or 10 in SD rats (Singh et al., 1975). The dams were killed at 24 hours interval starting on days 8 and 11 until day 20 of gestation. Radioactivity was detected in foetal tissues, amniotic fluid and placenta at all time points. The radioactivity peaked at 48 hours and declined rapidly thereafter. The concentration was less than that of maternal blood and less than 1% of the administered dose.

Mice

The distribution of (carbonyl-^{14}C)DEHP was studied in male CD-1 mice following intravenous injection by tail vein of 1 ml DEHP-enriched plasma (2,293 μg/ml) (Waddell et al., 1977). After 1, 3, 9, 24, and 168 hours, the mice were killed and subjected to whole-body autoradiography.

The radioactivity was rapidly accumulated in the kidney and liver, with with high concentrations in urine, bile, and intestinal contents. There was no evidence of retention in any tissues in the body. From the amount of radioactivity seen in the sequence of time intervals, it seems clear that the material is rapidly and completely eliminated by the kidney and the liver. The secretion by the liver, via the bile into the intestine appears to be the major route. No enterohepatic circulation of DEHP was indicated since there was a persistently high concentration in the intestinal lumen.

4.1.2.1.5 *In vitro* studies

The enzymatic hydrolysis of DEHP by different lipases was studied in tissues from the Sprague-Dawley rat (CD-strain), guinea pig, hamster and mice (Albro and Thomas, 1973). DEHP was hydrolysed to MEHP by lipases from a variety of rat tissues with pancreas, liver, and intestinal mucosa containing the bulk of the DEHP hydrolase activity. There was no difference in DEHP hydrolase activity in terms of units per mg of protein between intestinal homogenates from young adult (200g) and old (450g) rats, but there was higher activity from male than from female rats (1.7 and 0.45 units/g protein, respectively), and from fed than from fasted rats (1.7 and 0.74 units/g protein, respectively). Variation among species was not extreme, although different mouse strains showed considerable differences (e.g. 1.2 and 4.0 units/g protein for CD-1 and C57B1/6f, respectively). Out of 15 tissue preparations, only the liver alkaline lipase preparation was able to further hydrolyse MEHP to phthalic acid, at a rate of 2% at which it hydrolysed DEHP.

The activities of liver, lung and kidney of rats of various age group and that of placenta in hydrolyzing di(2-ethylhexyl) phthalate to mono(2-ethylhexyl) phthalate have been measured (Gollamudi et al., 1985). Male and female Sprague-Dawley rats of 45 days of age, neonatal rats within 12 hours of parturition, and fetuses and placenta on day 19 of gestation were used. The liver was most active in all age groups; however, the lung and the kidney also had considarable activity. The tissues of the fetuses and the neonate had significant activity. The Km values of the enzyme were 4, 1.25 and 5.9 mM, respectively, in the neonatal, adult and old livers (Gollamudi et al., 1983).

The absorption of DEHP and MEHP was studied using an everted gut-sac preparation form the male Sprague-Dawley (300-400 g) rat small intestine (White et al., 1980). MEHP was significantly less lipophillic than DEHP, and was absorbed by the everted gut sac in a significantly greater quantity than DEHP. Esterases within the mucosal epithelium hydrolysed DEHP quantitatively to MEHP.

Lake et al. (1977) studied the hydrolysis of DEHP in hepatic and intestinal preparations from various species. Hepatic postmitochondrial supernatant preparations from Sprague-Dawley rat, olive baboon, and albino ferret were able to hydrolyse DEHP to MEHP as well as were intestinal mucosal cell preparations from rat, baboon, ferret, and man. According to the authors, these results show a species similarity in the metabolism of DEHP between man, a rodent, a nonrodent, and a nonhuman primate species. Furthermore, the results suggest that orally ingested DEHP would most probably be absorbed from the gut of the rat, baboon, ferret, and man primarily as the corresponding monoester.

Gray et al. (1982) studied the hydrolysis of DEHP in intestinal preparations from young Sprague-Dawley rats (28-42 days) and Dunkin-Hartely hamsters. During a 16-h incubation, DEHP was hydrolysed to MEHP which was significanly different between rats (18.9 ± 2.1%) and hamsters (4.1 ± 1.0%).

The hydrolysis of DEHP was studied in hepatic post-mitochondrial supernatant fractions and in intestinal mucosal cell whole homogenates obtained from untreated Sprague-Dawley rats and Syrian hamsters (Lake et al., 1984b). Rat hepatic DEHP hydrolase activity was more than twice as active as the enzyme present in hamster liver. Rat intestinal DEHP hydrolase activity was thrice as active as the hamster intestinal activity. According to the authors, these differences may explain the different hepatic response of rats and hamsters to DEHP.

Two groups of 6 Sprague-Dawley rats which were 25 days old and two groups of 6 Sprague-Dawley rats which were 60 days old were used to study *in vitro* metabolism (Sjöberg et al., 1985c). One group of each age were pre-treated with DEHP (gavage, 1,000 mg/kg bw for 14 days) and the other group of each age were pre-treated with phenobarbital (intraperitoneal injection, 100 mg/kg bw for 3 days). After the animals were killed liver microsomes were prepared and concentrations of mono-(2-ethyl-5-hydroxyhexyl) phthalate was determined. The conversion of MEHP to mono-(2-ethyl-5-hydroxyhexyl) phthalate in liver microsomes from untreated 25- and 60-day-old rats were 0.37 ± 0.07 and 0.39 ± 0.08 nmol/mg protein/min, respectively. The rate of (ω-1) hydroxylation in liver microsomes from rats pretreated with DEHP was 0.33 ± 0.05 nmol/mg protein/min. Liver microsomes pretreated with phenobarbital showed a two fold increase in the conversion rate 0.73 ± 0.15 nmol/mg protein/min.

In another experiment the protein binding of MEHP in plasma from 25, 40 and 60-day old Sprague-Dawley rats at 25 μg/ml was determined using the equilibrium dialysis techqnique (Sjöberg et al., 1985c). The binding of MEHP to plasma proteins was 97.6 ± 1.5, 98.0 ± 0.2 and 97.5 ± 0.4% in the 25, 40 and 60-day age group, respectively. The plasma protein binding was constant in the concentration interval 5-150 μg/ml.

The hydrolysis rates of DEHP were measured in suspensions of contents of the Wistar rat stomach, small intestine or caecum (Rowland et al., 1977). After 16 hours, 1.0% of DEHP was hydrolysed by stomach, 22.1% by small intestine, and 6.9% by caecum contents. The hydrolysis product was identified as MEHP.

In studies reported by Lhuguenot et al. (1985), the metabolism of MEHP was studied in isolated and cultured rat (Wistar derived) hepatocytes. At concentrations of 50 or 500 μM (^{14}C) MEHP (position of label not stated, highest available purity), the substance was extensively metabolised. No water-soluble conjugates were detected. At the low concentration, recoveries of radioactivity of 71-79% were obtained. The amount of unchanged MEHP remaining in the medium increased slightly from day 1 to day 3. Metabolites I and V are final products of MEHP metabolism, no further metabolism was detected in this study. Metabolite X was transformed to metabolite V, which was further transformed to metabolite I, and metabolite IX was transformed to metabolite VI. A small increase in metabolite I and a decrease in metabolite VI were seen from day 1 to day 3. At the high concentration, recoveries of radioactivity ranged from 74 to 83%. The amount of unchanged MEHP decreased from 149 μM after 1 day to 93 μM after 3 days. A fivefold increase in metabolite I and a smaller increase in metabolite V along with time-dependent decreases in metabolites VI and IX were observed.

Accumulation of DEHP and MEHP in blood and blood components was studied during storing of whole blood, platelet-rich plasma and platelet-poor-plasma from 72 hours to 4 weeks (Rock et al., 1978). Both phthalates showed a progressive increase in concentration over time. While the levels of DEHP were much greater than those of MEHP, there was nonetheless a significant and continual increase in MEHP in all preparations. The highest concentrations of both DEHP and MEHP was found in platelet-poor-plasma, indicating that platelets do not have a major role in

the accumulation of phthalates in blood. The accumulation of MEHP was shown to be direct result of the metabolism of DEHP by plasma protein(s) rather than leaching from the blood bag.

4.1.2.1.6 Summary and Discussion

There are a limited number of studies on the toxicokinetic behaviour of DEHP in man. These reports concern exposure by the oral, intravenous and inhalatory routes, however, they contain limited information. A single study performed by the dermal route is not considered reliable. Toxicokinetic studies in animals have been performed by the oral, inhalatory, dermal and parenteral routes. The majority of these studies have, however, been performed by the oral route and in three different strains of rat. Several studies with non-human primates, including old world- and new world apes, and mice have been conducted. In addition, there are several studies including other species. By the parenteral routes has been most studied in non-human primates, but there are few studies by the dermal and inhalation routes. In addition, *in vitro* studies have also contributed to the understanding of the toxicokinetics.

The majority of the toxicokinetic studies as a whole are mainly research orientated and published in the open litreature.. Dose levels greatly varied between studies. The quality and relevance of these studies is variable and it was necessary to examine the database as a whole to derive conclusions.

Oral exposure

Numerous studies on the toxicokinetics following oral administration of DEHP have been performed in experimental animals, especially rats, whereas only a few studies with humans are available.

Absorption

The rate and extent of intestinal absorption of DEHP have been estimated indirectly by measuring urinary excretion of ^{14}C-DEHP-derived radioactivity as a function of time compared with the administered dose. There are, however, only a few reports that study ^{14}C levels in blood. Taken together these reports indicate that DEHP, probably as MEHP, is rapidly absorbed from the gastrointestinal (GI) tract following oral administration. Oral exposure to less than massive amounts of DEHP does not result in exposure of internal organs to intact DEHP since hydrolysis is very rapid in gut. In Fischer rats, as the dose increased, a threshold was reached, at about 450 mg/kg bw, above which there was a steady increase in the amount of unhydrolysed DEHP reaching the liver (Albro et al., 1982a). In contrast, an absorption threshold could not be determined in $C3B6F_1$ mice for doses up to 1,000 mg/kg bw. There are no data available concerning a possible absorption threshold in humans.

The extent of absorption in rats, non-human primates and humans is around 50% for doses up to about 200 mg/kg bw (see **Table 4.24**). At higher doses it appears that absorption in non-human primates is dose-limited in contrast to rodents (Rhodes et al., 1983). To determine the extent of uptake based on the amount recovered in the urine, excretion via other pathways (e.g. faeces, bile), enterohepatic reabsorption, and body retention have to be considered. In non-human primates, an i.v. correction factor of around 1:2 (50% bioavailability) for peroral exposure is considered valid (Rhodes et al., 1983). For rats an i.v. correction factor has not been determined but biliary excretion is low (ca.11%), and the subsequent amount that can be reabsorbed is not known. Hence, it is assumed that GI-tract absorption in rats may also bearound 50%. It is however, difficult to estimate the bioavailability in mice, since biliary excretion is probably high

and the extent of reabsorption is not known. Although there are only two studies by the oral route in humans it seems reasonable, based on the i.v. correction factor for non-human primates and compared with the absorption in animals that human uptake will also be around 50%. In conclusion, an oral bioavailability of 50% in rats, non-human primates and humans is considered valid if data on massive dose levels in non-human primates is excluded.

Observations suggest that the extent of absorption, and hence total systemic exposure to MEHP and its metabolites, is higher in young rats than in old when DEHP is administered by gavage (Sjöberg et al, 1985c). The reported relatively higher proportion of intestinal tissue in relation to body weight (Younoszai and Ranshaw, 1974, 1973), and the relatively higher blood flow through the gastro-intestinal tract (Varga and Csaky, 1976) may be factors causing an increased absorption in young animals. Other factors involved in an age-related difference in absorption, such as small instestinal propulsion motility and gastric emptying time have not varied between 20-day old rats and mature rats (Varga 1976, 1975). Furthermore, reports on the influence of age on intestinal esterase (Holmes and Masters, 1967) and pancreatic lipase activities (Larose and Morisset, 1977) indicate that the hydrolysis of DEHP is not higher in 25-day-old than in mature rats. Apart from kinetic differences, variation in tissue sensitivity may be involved in the age-related response.

Distribution

Distribution studies have mostly measured the distribution of radioactivity from (^{14}C) DEHP in tissues of laboratory animals. In most cases only total radioactivity has been measured with no distinction between DEHP and its metabolites, so little is known about tissue distribution of DEHP and its metabolites.

The studies available indicate that the radioactivity from (^{14}C) DEHP is widely distributed in the tissues without evidence of accumulation in the tissues of rat (Pollack et al., 1985a and Daniel and Bratt, 1974). A comparative study in rats and marmosets showed similar distribution patterns of ^{14}C in the two species after oral administration, even though rats had higher tissue levels than marmosets (Shell, 1982 and Rhodes et al., 1986). Thus, the difference in distribution between species is quantitative rather than qualitative. Clearance of unlabelled DEHP from renal fat, muscle, heart and lungs in pigs, and from muscle, skin adipose tissue and liver in broiler hens was apparently low; however, a limited number of animals were used in these studies (Jarosová et al., 1999).

Following single oral absorption, the radioactivity in mice was widely distributed in organs and tissues without evidence of tissue storage (Gaunt and Butterworth, 1982). The amount of radioactivity in the brain was low, especially in 10- and 20 days-old mice, and retention of radioactivity in the brain was minimal (Eriksson and Darnerud, 1985). The amount of radioactivity in the liver was about 10 times that in the brain. After 24 hours the amount of radioactivity found in the livers ranged from about 27 to 2% in the order 3-, 10- and 20-day old mice, showing significant decreases in all ages after 7 days.

In one study DEHP was detected in the livers of pups from mothers given DEHP through the lactation period, indicating that DEHP can be transferred through the milk (Parmar et al., 1985). This was supported by another study where DEHP and MEHP were secreted into the milk of lactating rats (Dostal et al., 1987a).

Table 4.24 Data used to estimate the oral bioavailability for different species and strains

Species /strain	Dose, mg/kg bw/day (duration)	Recovery Urine (%)	Faeces (%)	*i.v.* correcetion	Oral Bioavailability[1]	Reference
Rat: Sprague-Dawley rat	100 (single)	51	43	ND	approximately 50%	Lake et al., 1984b
	1000 (single)	ND	53			
	100 (single)	30	61	ND	30%	Eastman Kodak Co., 1983; 1984a
	10 (single)	54	43	ND	approximately 50%	General Motors 1982ab
	50 (ca. 28 days)	37.5	53	ND	approximately 40%	Ikeda et al, 1980
Wistar	2.9 (single)	42	57	ND	approximately 50%	Daniel and Bratt, 1974
	50 (3 days)	50	ND	ND	approximately 50%	Lhuguenot et al., 1985
	500 (3 days)	60	ND		approximately 50%	
Fischer 344	85 (single, 7 or 21days)	53	37	ND	approximately 50%	CMA, 1982a; Lington et al., 1987; Short et al., 1987; Astill et al., 1986
	550 (single, 7 or 21days)	64	28	ND	>50%	
	1000 (single, 7 or 21days)	68	25	ND	>50%	
Monkeys	100 (single)	28	49	50%	42%	CMA, 1982b. 1983, 1984a; Short 1987; Astil 1986
Cynomolgus	100 (21days)	20 and 55	49 and 39	50%	approximately 50%	Short etal, 1987; Monsanto, 1988
	500 (21days)	4 and 13	69 and 56	50%	approximately 6%	
Marmoset	100 (single)	20-40	25	50%	approximately 45%	ICI 1982a; Shell 1982; Rhodes 1983, 1986
	2,000 (single)	4	84	50%	6%	Rhodes 1983
	2,000 (14 days)	0.9 (M) and 1.3 (F)	59 (M) and 72 (F)	50%	approximately 2%	
Human	30 mg (single)	15	ND	50%[2]	37.5%	Schmid and Schlatter, 1985
Volunteers	10 mg/d (4 days)	25	ND	50%[2]	37.5%	Schmid and Schlatter, 1985
	213 mg (single)	31	ND	50%[2]	46.5%	Bronsch, 1987
	70 mg/d (3 days)	27	ND	50%[2]	40.5%	Bronsch, 1987

1) Corrected for *i.v.* correction factor in non-human primates and humans
2) Based on monkey studies
ND) Not Determined

Available data indiacte that DEHP can cross the placental barrier since DEHP was detected in rat foetal livers after feeding DEHP to the dams during gestation (Srivastava et al., 1989 and Singh et al., 1975) and radioactivity was detected in the embryo after given (^{14}C)DEHP to pregnant mice (Lindgren et al, 1982). A high concentration was also seen in the uterine fluid. Except for the gut and the neuroepithelium, uptake in the embryo was low. At late gestation, a very high accumulation was seen in the yolk sac after oral administration of either (2-ethylhexyl-1-^{14}C) DEHP or (carbonyl-^{14}C) DEHP. The distribution in the fetuses was very similar after administration of the two ^{14}C-labelled DEHP compounds.

Metabolism

The first step in the metabolism of DEHP is hydrolysis to MEHP and 2-ethylhexanol (see

Figure 4.1). Oral exposure to less than massive amounts (below 200 mg/kg bw) of DEHP does not result in exposure of internal organs to intact DEHP since DEHP is rapidly hydrolysed in the intestines. The enzymes responsible for the hydrolysis of DEHP to MEHP *in vitro*, the lipases, are found in all tissues surveyed (intestinal mucosa, liver, kidney, lungs, skin, pancreas and adipose tissues) but especially in the pancreas, indicating that most of DEHP metabolism occurs in the lumen of the small intestine (Albro and Thomas, 1973; Daniel and Bratt, 1974). Lake et al. (1977) concluded that the hydrolysis of phthalates is qualitatively similar between the rat, the ferret, the baboon and humans, though it may differ somewhat quantitatively. Data from Albro and Thomas (1973) indicate that the lipase activity is highest in the intestines of the mouse followed by the rat, guinea pig and hamster. In addition the DEHP lipase acivity was higher in males than in females, and higher in fed animals than in fasted. De-esterfication of DEHP absorbed from the GI-tract occurs in the liver and blood by lipases (Albro and Thomas., 1973).

MEHP is not hydrolysed to phthalic acid by the enzyme hydrolyzing DEHP. Only one tissue preparation out of 15 was able to hydrolyse MEHP to phthalic acid at 2% of the rate at which it hydrolysed DEHP (Albro and Thomas, 1973). Instead MEHP is further metabolised *in vivo* in the liver by oxidation (Albro et al., 1984). The oxidative metabolism of MEHP begins with hydroxylation of the ethylhexyl side chain at five different positions resulting in the formation of primary (ω-oxidation) and secondary ((ω-1)- and (ω-2)-oxidation) alcohols. These alcohols are then oxidised to diacids (ω-oxidation) or diketoacids ((ω-1)-oxidation), respectively. The diacids are apparently subject to α- or β-oxidation at the ethyl- or hexylchain, respectively, in mitochondria and peroxisomes to yield shorter diacids. This process does not extend beyond the branch point in the ethylhexyl-chain. It has been reported that all of the metabolites in urine of (^{14}C)DEHP are also found in urine of rats given

Figure 4.1 The first step in the metabolism of di-(2-ethylhexyl)phthalate DEHP is hydrolysis by lipases to mono-(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanol (2-EH)

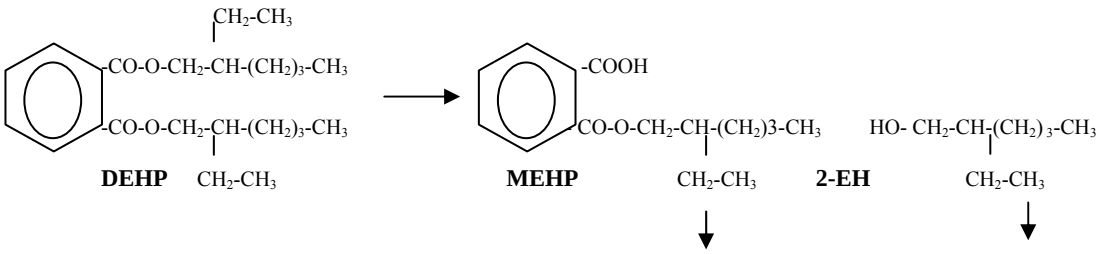


Table 4.25 Structure of DEHP metabolites, adopted from Albro et al., (1983).

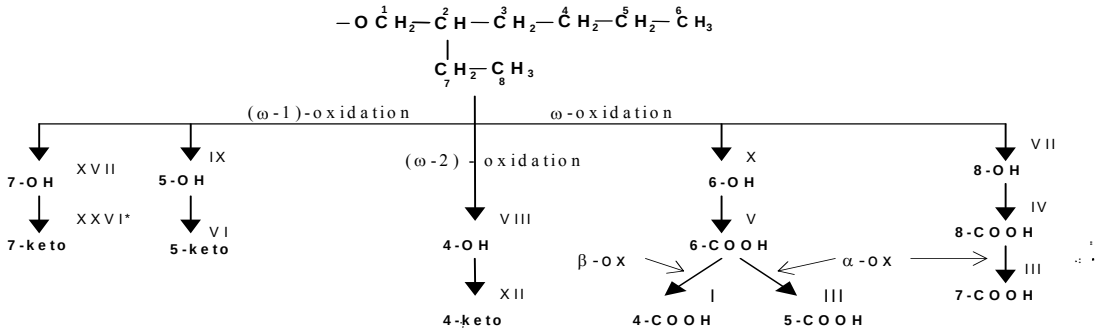
HOOC-C_6H_6-$COOCH_2$-CH-*R'* | ***R''***			
Metabolite		**R'**	**R''**
I	2-ethyl-3-carboxypropyl phthalate	-CH_2COOH	-CH_2CH_3
II	2-carboxyhexyl phthalate	-$[CH_2]_3CH_3$	-COOH
III	2-ethyl-4-carboxybutyl phthalate	-$[CH_2]_2COOH$	-CH_2CH_3
IV	2-carboxymethylhexyl phthalate	-$[CH_2]_3CH_3$	-CH_2COOH
V	2-ethyl-5-carboxypentyl phthalate	-$[CH_2]_3COOH$	-CH_2CH_3
VI	2-ethyl-5-oxyhexyl phthalate	-$[CH_2]_2$-CO-CH_3	-CH_2CH_3
VII	2-(2-hydroxyethyl)hexyl phthalate	-$[CH_2]_3CH_3$	-CH_2CH_2OH
VIII	2-ethyl-4-hydroxyhexyl phthalate	-CH_2-CHOH-CH_2CH_3	-CH_2CH_3
IX	2-ethyl-5-hydroxyhexyl phthalate	-$[CH_2]_2$]-CHOH-CH_3	-CH_2CH_3
X	2-ethyl-6-hydroxyhexyl phthalate	-$[CH_2]_3CH_2OH$	-CH_2CH_3
XI	2-ethyl-pentyl phthalate	-$[CH_2]_3CH_3$	-CH_2CH_3
XII	2-ethyl-4-oxyhexyl phthalate	-CH_2-CO-CH_2CH_3	-CH_2CH_3
XIV	2-carboxymethyl-4-oxyhexyl phthalate	-CH_2-CO-CH_2CH_3	-CH_2COOH
XV	2-ethyl-4-oxy-6-carboxyhexyl phthalate	-CH_2-CO-CH_2COOH	-CH_2CH_3
XVI	2-ethyl-4-hydroxy-6-carboxyhexyl phthalate	-CH_2-CHOH-CH_2COOH	-CH_2CH_3
XVII	2-(1-hydroxyethyl)hexyl phthalate	-$[CH_2]_3CH_3$	-CHOH-CH_3
XVIII	2-carboxymethyl-4-hydroxyhexyl phthalate	-CH_2-CHOH-CH_2CH_3	-CH_2COOH
XIX	2-(1-hydroxyethyl)-5-hydroxyhexyl phthalate	-$[CH_2]_2$-CHOH-CH_3	-CHOH-CH_3
XX	2-ethyl-4,6-dihydroxyhexyl phthalate	-CH_2-CHOH-CH_2CH_2OH	-CH_2CH_3
XXI	2-carboxymethyl-5-hydroxyhexyl phthalate	-$[CH_2]_2$-CHOH-CH_3	-CH_2COOH
XXV	2-carboxymethyl-5-oxyhexyl phthalate	-$[CH_2]_2$-CO-CH_3	-CH_2COOH
XXVI	2-(1-oxyethyl)hexyl phthalate	-$[CH_2]_3CH_3$	-CO-CH_3

(^{14}C)MEHP) (Albro et al., 1983), and at least 98% of the ^{14}C present in urine of rats given carbonyl-^{14}C labelled DEHP either orally or *i.v.* has been accounted for in the form of some 20 metabolites (see **Table 4.25**), none of which retained both ester functions. **Figure 4.2** illustrates the most important pathways to which MEHP is subjected, and the resulting metabolites named according to the widely accepted nomenclature of Albro et al., (1983). Only the side chain is shown since there appears to be no alteration of the aromatic ring in mammals (Albro et al., 1983).

In **Table 4.26** an attempt is made to illustrate the distribution of metabolites in urine in different species after exposure to DEHP. MEHP is a relatively major component in urine of monkeys, guinea pigs and mice but was mostly not detected in rat urine. However, in plasma MEHP is present in all species tested. The plasma concentrations and mean AUC's for each of the MEHP-derived metabolites were considerably lower than those of MEHP both after single and after repeated administration, and the maximal plasma concentrations and mean AUC's did not differ significantly between animals given single or repeated doses of DEHP (Sjöberg et al., 1986a,b). MEHP is apparently quite stable since ^{14}C-MEHP in plasma increased to a plateau within a few

minutes and then remained at a constant level for more than 30 minutes even though the total ^{14}C-plasma levels decreased (Albro et al., 1981 and Peck and Albro, 1982).

Figure 4.2 Metabolites from DEHP and MEHP excreted into the urine. Only the ethylhexyl chain of MEHP and its metabolic alterations are shown. Metabolite identified by asterix (*) has not been detected



Oxidative metabolism of MEHP plays a dominant role in the rat after oral administration, with approximately 75% of urinary metabolites consisting of dicarboxylic acids derived by ω-oxidation of the ethylhexyl chain, whereas after *i.v.* administration there seem to be a more even distribution between ω- and (ω-1)-oxidation (see **Table 4.26**). The predominant metabolites in non-human primates are MEHP and metabolite IX (a secondary alcohol) derived by (ω-1)-oxidation. MEHP was converted to metabolite V in the monkey but, in contrast to the rat and mice, further oxidation to metabolite I was negligible. Metabolite I was a major component in both rat and mouse urine, while metabolite V was a major component in rat urine but a negligible component in mouse urine. Thus, it appears that ß-oxidative metabolism of DEHP is a major pathway in rodents but not in primates. With regard to DEHP metabolism the mouse is somewhere in between rats and non-human primates. ß-oxidative metabolism of DEHP is a major pathway in rodents but not in non-human primates, whereas high levels of MEHP and metabolites from (ω-1)-oxidation are major metabolites in urine from non-human primates and mice, but not from rats. The limited human data indicate that human DEHP metabolism resembles that of other primates and mice (except for the presence of metabolite I and phthalic acid in the mice). The similarity with the mice is supported by high levels of phthalic acid observed in urine from patients undergoing maintenance hemodialysis for the treatment of renal failure (Pollack et al., 1985b**)**. However, it is not possible to draw any conclusions about the relevance for the general population since this observation might be related to renal insufficiency. In addition, phthalic acid was a main metabolite in urine from Cynomolgus monkeys orally exposed to DEHP (Short et al., 1987 and Monsanto, 1988).

Enzyme induction was indicated after repeated doses in rats at high doses (i.e. increase in the urinary excretion of ω-/β-oxidation products and decrease in (ω-1)-oxidation products), but little or no alteration was observed in urinary metabolite profile after exposure to low doses (Lhuguenot et al., 1985).

Table 4.26 Example of distribution of metabolites in urine after administration of DEHP*

		Single Oral				Single i.v.		
Oxidation	**Metabolite**	**Rat 180 mg/kg**	**Mouse 360 mg/kg**	**Guinea pig 180 mg/kg**	**Hamster 20 mg/kg**	**Rat**	**Green Monkey**	**Man**
	DEHP	-	0.5(0)	-	0.3(0)		2.2	-
	MEHP	-	18.6(79)	71.2(74)	4.5(39)	1.9	28.9(90)	18.3
	PA	-	12.4(29)	5.4(55)	9.5(14)	1.84	Trace	0.1
ω hexyl	X	0.6	2.2(63)	1.3(72)	1.9	0.23	0.7(94)	0.1
ω hexyl	V	51.3	1.1	6.9	14.0	24	4.2(0)	5.3
ω hexyl, β	I	17.2	16.8	2.4	13.0	5.1	Trace	-
ω hexyl, α	III	1.2	0.4(45)	0.5(16)	0.3(20)	0.9	-	-
ω ethyl	VII	2.6	7.2(77)	0.8(23)	4.9	3.9	7.0(72)	11.9
ω ethyl	IV	3.3	0.8	0.4	0.4	4.0	-	1.2
ω ethyl, α	II	2.0	1.0	0.4	0.1	0.9	-	1.8
(ω-1)hexyl	IX	13.3	12.3(70)	3.4(50)	32.7(11)	18	38.2(85)	36.2
(ω-1)hexyl	VI	2.6	14.9(81)	1.1(0)	10.2(6)	18	5.9(62)	12.1
(ω-2)hexyl	VIII	-	-	-	-	0.35	5.7(74)	8.1
	Other	1.8	11.5	6.2	6.1		7.6	4.9

* Adapted from Albro et al., 1981; 1982a; 1982b; 1983; Peck and Albro, 1982. Data are based on ^{14}C-distribution and expressed as percentages of total metabolites. Urine was hydrolysed with β-glucuronidase for the data. Numbers in brackets are the percentage of the metabolite in the form of glucuronide ester conjugate (Albro et al., 1981; 1982b). It should be noted that urine was collected from several sources after single oral (rat, mice, hamster, and guinea pig) or single *i.v.* (Sprague-Dawley rat, African Green monkey and human leukemia patients) administration.

According to Egestad et al. (1996), and in comparison with data from earlier studies in adults, there is a somewhat higher capacity for oxidation of MEHP in newborns. In addition, metabolite VIII ((ω-2)-oxidation) was found to be more abundant than metabolite VII (ω-oxidation) in urine from old rats (Albro et al, 1983). Metabolite VIII had before that only been observed in urine from primates. Whereas younger rats given (7-^{14}C)DEHP excrete 40-50% of the radioactivity in the urine in 24 hours (Albro et al., 1973), these old rats excrete only 10-15% in that time interval, suggesting that the relative accumulation of minor metabolites may depend upon the efficiency of elimination.

The metabolites of DEHP can be conjugated before excretion. Glucuronidation is the major conjugation pathway for the metabolites of DEHP in the investigated species (see **Table 4.27**). In addition, approximately 3% of the administered dose in mice were found in the urine as ß-glucose conjugate (Egestad et al., 1996). The ß-glucose conjugate was not observed in the guinea pigs. Species differences have also been observed for glucuronides since conjugates of glucuronic acid were absent in rats, low (15% of excreted metabolites were glucuronides) in hamsters, moderate (60-65%) in mice and guinea pigs. In primates, including humans, about 80% of the metabolites were glucuronides after intravenous administration (Albro et al., 1982). Following oral adminstration about 65% of the metabolites were glucuronides in two volunteers (Schmid and Schlatter, 1985), whereas in another study 99% were glucuronide conjugates (Bronch, 1987). When urine from 5 workers occupationally exposed to DEHP by inhalation was analysed (Dirven et al., 1993b) metabolites IX and VI were almost completely conjugated in all five persons (IX: 88-100% conjugated; VI: 80-95% conjugated) while 32-45% of metabolite V

was present in conjugated form. For MEHP, a large inter-individual variation was found with 77% and 100% present in free form in two persons but only 20, 30 and 38% in the other three persons. In urine from an infant exposed to DEHP by exchange transfusion the fraction of metabolites conjugated with glucuronides was dominated by several metabolites from DEHP but no conjugated MEHP was found (Egestad et al., 1996). From available data (Dirven et al, 1983b; Albro et al., 1981; 1982b) it is obvious that conjugation to glucuronic acid occurs both for MEHP and for metabolites oxidised by ω-, (ω-1)-, and (ω-2)-oxidation (see also **Table 4.26**). In addition, the data produced by Dirven et al. (1983b) indicate that there are substantial inter individual differences in the glucuronidation of some metabolites of DEHP, and of MEHP in particular. One possible explanation to this might be a polymorph distribution of ß-glucuronidase within the population.

Table 4.27 Forms of excretion products in urine from different species, expressed as percentage of total excretion products*

Species	Route	Free	Conjugated	Reference
Rat, three strains	oral	100	0	Albro et al., 1982a
Hamster	oral	85	15	Albro et al., 1982a
Mouse, CD	oral	36	64	Albro et al., 1982a
Guinea pig	oral	35	65	Albro et al., 1982a
Man	oral	35	65	Schmid and Schlatter, 1985
Green monkey¤	*i.v.*	20	80	Albro et al., 1982a
Man	*i.v.*	20	80	Albro et al., 1982a
Man¤$	oral	1	99	Bronch, 1987

* Urine was hydrolyzed with β-glucuronidase for the data. (Albro et al., 1981; 1982a; Peck and Albro, 1982; Bronch, 1987; Schmid and Schlatter, 1985). It should be noted that urine was collected from several sources after single oral (rat 180, mice 360, hamster 20 and guinea pig 180 mg/kg bw) or single *i.v.* (African Green monkey and human leukemia patients) administration.

Glucuronide conjugates are substrates to β-glucuronidase that is present in the lyzosomes of some mammalian tissues and in the intestinal microflora. This enzyme can release the aglycone, which can be reabsorbed and enter the enterohepatic recirculation. Compounds involved in this cycle tend to have a longer lifetime in the body and may undergo more extensive biotransformation before being excreted. Following intravenous administration of 3.5 mg/kg (^{14}C) MEHP to Sprague-Dawley rats up to 52% of the radioactivity entered the intestine from the bile, but only 8% of the dose was excreted in faeces (Chu et al., 1978). This would indicate that resorption of radioactivity took place in the intestine of rats. The rise in the radioactivity in the blood observed after the rapid decrease could be attributed to the reabsorption of biliary secreted material (Chu et al., 1978). A secondary rise in blood DEHP concentration was also observed in another study on Sprague-Dawley rats after oral, intraperitoneal or *i.v.* dosing (Pollack et al., 1985). Apparently MEHP or its metabolites is reabsorbed in the intestine of rats. Data on the fraction of DEHP and its metabolites present in the bile is not available. Unfortunately, there is no data available about enterohepatic circulation of DEHP from other species than the rat. In mice a persistently high concentration in the intestinal lumen was observed which might indicate that no enterohepatic circulation occurred (Waddell et al., 1977).

In addition, to the 20 known metabolites of DEHP, derived from MEHP and retaining in the o-phthalate moiety, at least 7 metabolites produced by rats from 2-ethylhexanol have been identified (Albro et al., 1975). The carbon chain of 2-ethylhexanol is ultimately capable of being metabolized in the liver to CO_2 and acetate through oxidative pathways (ω- and (ω-1)- oxidation with subsequent β-oxidation) (Albro 1975; Albro et al., 1982b). About 6-7% of orally administered 2-ethylhexanol was exhaled by rats, while about 10 and 80% was excreted in faeces and urine, respectively (Albro, 1975). Following oral administration of a single dose of 100 mg DEHP/kg bw rats expired 4% of the radioactivity as $^{14}CO_2$, 30% of the dose was excreted in the urine, and 62% was excreted in the faeces (Eastman Kodak Co., 1983), and around 28% of the dose remained in the lungs with minimal levels in other tissues after *i.v.* administration in rats (Rhodes et al., 1983).

Excretion

Generally, following oral administration of DEHP the substance and its metabolites are excreted rapidly and extensively in urine and in faeces. There is limited evidence of retention in organs and tissues.

After pre-treatment of rats elimination of ^{14}C was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete within 4 days (Ikeda et al., 1980). Faecal elimination was predominant in rats and dogs (53 and 75%, respectively) and urinary excretion (79%) was predominant in pigs. No more than traces of unmetabolised DEHP was found in urine from the three species (Ikeda et al., 1980). The ratio between radioactivity in urine and faeces after oral exposure to 2.9 mg/kg bw was about 40 and 60%, respectively, in urine and faeces. Following repeated exposure the ratio was changed to about 60 and 40%, respectively, in urine and faeces.

Only 10-15 percent of a single oral dose was eliminated in the urine of two volunteers exposed to 30 mg DEHP (Schmid and Schlatter, 1985), while 31% of the dose was recovered as DEHP metabolites in the urine within 47 hours following a single oral dose of 213 mg (Bronch, 1987). More than 53% of the recovered dose was eliminated the first 4 hours after exposure and 90% within 24 hours. After repeated exposure 15 and 25% of the administered dose was eliminated in the urine of the volunteers, and this excretion of metabolites showed strong daily fluctuations (Schmid and Schlatter, 1985). The daily fluctuations, may be associated with fluctuations in the diet. It should be noted that a great variability in metabolism and excretion also was observed between individuals of cynomolgus monkeys, both in the amount of the dose excreted in urine and in the metabolites identified.

Urinary excretion of DEHP occurred mostly within the first 24 hours, and a urinary elimination half-life of about 12 hours was estimated (Schmid and Schlatter, 1985). This short elimination half-life indicates that accumulation of DEHP in the human body is unlikely to occur.

Table 4.28 Quantitative distribution of metabolites of DEHP in urine of humans, expressed as percent of the four main metabolites

Metabolite		Workers[1]	Volunteers[2]	Patients[3]
		%	%	%
MEHP		26	10	25
IX	(ω-1, hexyl)	34	32	50
VI	(ω-1, hexyl)	18	24	17
V	(ω, hexyl)	22	33	7.4

1) Inhalation, n = 15, exposure unknown; Dirven et al, 1993b
2) 30 mg DEHP as a single oral dose, n = 2; Schmid and Schlatter, 1985
3) Intravenous, 95 and 174 mg to leukemia patients, n = 2; Albro et al, 1982

In another oral study the urinary elimination was considered to be biphasically, with an initial half-life of 10 hours. The half-life of the terminal phase was estimated to 22 hours.

The only striking difference in urinary excretion between oral exposure of volunteers and intravenous administration to leukemia patients is an increase in carboxylic derivative of the 2-ethylhexyl moiety in the volunteers (see **Table 4.28**). That means that the relative amount of metabolite IX in urine from leukemia patients was higher compared to volunteers, while the relative amount of metabolite V was lower (Albro et al., 1982b; Schmid and Schlatter, 1985; Dirven et al., 1993b). The difference could be associated with the form of administration (oral versus intravenous) or with the dose 30 versus 95 and 174 mg. However, it should also be recognized that the data on humans exposed to DEHP intravenously are from patients suffering from leukemia, and these patients have been on therapeutic regimens involving a multiplicity of drugs and treatments.

Many of the studies do not have complete recoveries, indicating that either biliary excretion and/or retention occur. Since many studies reveal that no significant retention was found in organs and tissues, biliary excretion might be an important excretion route. In the literature it is reported that the preferred route of excretion for glucuronide conjugates with molecular weight above 350 is the bile, while the preferred route of elimination for substances with molecular weight below 250 is in the urine (Klaassen, Amdur Doull, 1986). One study reveal that bile samples from Beagle dogs, and to a lesser extent from miniature pigs, accounted for a significant amount of administered ^{14}C dose, while less than 1% of the administered ^{14}C dose was secreted in the bile from bile duct cannulated Sprague-Dawley rats (Ikeda et al., 1980). According to Waddell et al., (1977) there are indications that secretion by the liver, via the bile into the intestine appears to be the major route in mice. The rates and extent of urinary and faecal excretion varied widely among monkeys, and data on volunteers indicate a great inter individual variability also among humans. About 5-10% of the dose in Wistar rats was recovered from the bile in 24 hours after oral administration of DEHP, whereas about 24%, of the dose was recovered from the bile after intravenous administration of DEHP (Tanaka et al., 1975 and Daniel and Bratt., 1974). From intravenous administration of MEHP the radioactivity secreted in the bile was 40%, at the higher exposure level (35 mg/kg) compared to 52% at the lower exposure level (3.5 mg/kg) (Chu et al., 1978). The implication for human excretion is not known.

Bioavailability

Based on experimental information derived from studies with rats, non-human primates and adult humans, oral absorption is around 50%. In addition, hepatic first pass metabolism is not considered to limit the systemic bioavailability. Therefore, the oral bioavailabilty for adults is

considered to be 50%. However, it cannot be ruled out that oral uptake of lower amounts of DEHP, similar to the actual human exposure levels, may be completely absorbed. Hence, 50% oral bioavailability is considered to represent a Reasonable Worst Case estimate.

There are limited data on the oral uptake of DEHP in young animals (Sjöberg et al., 1985c) and no information is available concerning infants or children. In addition, the oral uptake mechanism may depend on physiological, biochemical, and genetical age-dependent differences between adults and the young. Therefore, a precautionary approach is deemd warranted and a 100% oral bioavalability is considered a Reasonable Worst Case estimate for infants and children.

Inhalation

Absorption, Distribution, Metabolism and Excretion

The limited data regarding metabolism and excretion of DEHP in humans or animals following inhalation exposure indicate that no obvious difference should be expected when compared to oral administration.

Studies available on inhalation exposure to humans are not strictly toxicokinetic studies but case studies of patients (Roth et al., 1988) and worker (Liss et al., 1985; Dirven et al., 1993a and Dirven et al., 1993b). In workers exposed to DEHP by inhalation the main metabolites were: MEHP (26%), metabolites V (22%), VI (18%) and IX (34%). Metabolites IX and VI were almost completely conjugated in all five persons while 32-45% of metabolite V was present in conjugated form. For MEHP, a large human inter-individual variation was found with 77% and 100% present in free form in two persons but only 20, 30 and 38% in the other three persons. No substantial inter-individual variation in the phase I metabolism of DEHP was found. The absolute concentration of metabolites VI and IX was 1.7 times as high as the concentration of metabolite V supporting that (ω-1)-oxidation is favoured in humans.

In rats exposed to an aerosol of DEHP the major route of elimination was urine (General Motors, 1982ab). Within 72 hours following both single and repeated exposure, urine and faeces accounted for about 90% of the radioactivity, with around 50% in the urine and 40% in the faeces. The high excretion in faeces could be explained by excretion via the bile, but it is also probable that in this study that administered DEHP was also ingested, since approximately 75% of the aerosol retained in the animals was recovered in the lungs, and the remainder in the upper airways and gastro-intestinal tract. Hence, the observed excretion profile probably represents the contribution of oral and inhalatory absorption together. The study indicates that following repeated inhalation exposure long-term retention do not occur, and that the excretion profile following single exposure was faster initially but about 24 hours after exposure the urinary excretion was parallel following single and repeated exposure.

Bioavailability

Inhalation exposure to aerosols and particle bound DEHP are considered to be quantitatively the most important forms of DEHP absorption and exposure. DEHP vapour/gas is not considered separately. Adequate animal data in a relevant animal model are not available. The systemic bioavaibility following inhalation of DEHP will depend on different factors: 1) The size of the aerosol – this may vary from a fine mist to large particles depending on the occupational activity. Data on the aerosol and/or particle size are few and not sufficient to derive any quantitative estimates of the fraction bioavailable to the lungs. 2) Only a fraction of the amount inhaled will be available to the lungs while the majority will probably be swallowed and become orally

bioavailable. Based on the above mentioned oral bioavailability (discussed above), this fraction will be 50% in adults and 100% in infants and children. In addition, adequate data are not available on the size of the aerosol/particles, and bioavailability in the respiratory system. Hence, it is considered appropriate to use two different default value of 75% and 100% for adults and children, respectively.

Dermal exposure

Absorption, Distribution, Metabolism and Excretion

Dermal absorption studies have been performed both *in vivo* and *in vitro* where DEHP has been applied directly to the skin surface in all studies except for Deisinger et al., (1998). In that study a PVC film containing DEHP was secured to rat skin. For those studies in which DEHP has been applied directly different doses have been used. This is important since the magnitude of the percutaneous absorption rate is interdependent on the concentration of chemical in contact with the dermal surface ($J = K_p \times \Delta C$; where K_p is the permeability coefficient).

For several studies the percutaneous absorption rate has been derived based on the information detailed in the individual studies. In each case, the amount of (^{14}C) DEHP potentially bioavailable to the systemic system has been calculated as the combined amount of (^{14}C) DEHP in the excretion (urine and faeces), site of skin application i.e. residual (reservoir) (determined after washing and rinsing), and the fraction retained in the body (minus residual skin fraction). Not all *in vivo* studies determined the residual fraction remaining in the skin of rats and guinea pigs (i.e. Elsisi et al., 1989, Melnick et al., 1987 and Ng et al., 1992). Hence, the derived percentage percutaneous absorption for these studies represent a lower bound. The magnitude by which the percutaneous absorption is underestimated following a single application of DEHP will, in general, depend on: dermal contact time; concentration of chemical in contact with the skin; and amount bound to the skin. Chu et al (1996), included the skin bound dose when estimating the absorption in guinea pigs. The calculated cumulative amount detected in excreta and tissues (excluding the dosed skin was 6.5% (rat: Elsis et al., 1989), 9% (rat: Melnick et al., 1987), 26% (guinea pig: Ng et al., 1992), and including the skin bound dose 9.7-18.9% (guinea pig: Chu et al., 1996). Based on the results of the different *in vivo* studies, it is concluded that a reasonable value for *in vivo* absorption (potentially absorbed dose) of DEHP in animals will be around 20%. One study determined the percutaneous absorption rate (0.24 $\mu g/cm^2$/hour) for DEHP in PVC applied to rat skin (Deisinger et al., 1998).

In general the *in vitro* studies lack sufficient validation. Many variables are expected to influence results obtained with these types of studies e.g. vehicle used, receptor fluid etc. and standardised approaches are not currently recommended. Indeed, different results were obtained for diffusion cell experiments with epidermis skin discs from rats using a diffusion cell system and with DEHP (Scott et al., 1989 1987 and Pelling et al., 1998). Pelling et al., (1998) showed that the DEHP absorption rate for DEHP was increased (40-fold) when a 50% aqueous ethanol solution was used as receptor fluid compared to phosphate buffer. However, the permeability constant for very similar conditions (the 50% aqueous ethanol system) was also greatly different higher (16-fold) when compared with the results of Scott et al. (19891987). Difference in experimental conditions was suggested to explain this latter result. Therefore, it is considered difficult to judge the validity and representativeness of these studies when making conclusions about quantitative values for risk assessment.

The *in vitro* results of Scott et al. (1989, 1987) and Barber et al. (1992), however, indicate that rat skin is 4-fold more permeable than human skin. This seems a valid assumption for the

application of high amounts of DEHP as this difference was an intra-study observation. Hence, based on the assumption that humans absorb 4-fold less DEHP than rats when exposed to undiluted DEHP.

Bioavailability

Based on in vivo studies in animals the cumulative bioavailbility of DEHP is 20%. Based on the *in vivo* data, and application of a across-species correction factor of 4 a dermal absorption value of 5% is considered reasonable for potential human percutaneous absorption. A dermal bioavailability of 5% is also used for exposure to DEHP contained in PVC products because the migration coefficient of DEHP from the product has also to be considered. Each stage is, therefore, clearly defined. Hence, 5% is used for both exposure to free DEHP and DEHP contained in plastic products.

Other Routes

Absorption and Bioavailability

The limited data regarding metabolism and excretion of DEHP in humans or animals following intravenous administration indicate that no obvious difference should be expected when compared with oral administration. When exposure to DEHP occurs via the parenteral route uptake is 100% and because hydrolysis by intestinal lipases is bypassed and the liver is not a first-pass organ as for oral exposure, the amounts of intact DEHP in the organs and tissues would be expected to be higher.

It should be recognised that data on humans exposed to DEHP *i.v.* are mostly from patients undergoing chronic haemodialysis or suffering from serious diseases like leukaemia, renal failure and cancer (Pollack et al., 1985b, Malik et al., 1993 and Peck and Albro, 1982). These patients have been on therapeutic regimens involving a multiplicity of drugs and treatments, and it will not be possible to compare the DEHP metabolite distribution of man and laboratory animal unless data on normal healthy humans become available. There are also data from infants exposed to DEHP during exchange transfusion (Sjöberg et al., 1985 a,b and Egestad et al., 1996). The immature livers of new-born infants may have a lower metabolising capacity than that of older children and adults. Thus, these patients might be especially susceptible to possible harmful effects of DEHP and MEHP.

Distribution

The distribution was similar following administration of (2-ethylhexyl-1-^{14}C)DEHP and (carbonyl-^{14}C)DEHP intravenously to mice (Lindgren et al., 1982). Four hours after intravenous injection, a very high activity was observed in the gall bladder, intestinal contents and urinary bladder, a high uptake was also seen in the liver, kidney, and brown fat. Twenty-four hours after administration the activity in the gall bladder, intestinal contents and urinary bladder was still very high. This support the theory that secretion by the liver, via the bile into the intestine appears to be the major route in mice.

Parallel decreases in the plasma concentrations of DEHP, MEHP and metabolites V, VI and IX in rats, after single infusion of DEHP indicated that the elimination of DEHP was the rate limiting step in the disposition of the metabolites (Sjöberg et al., 1985d). This was partly supported by the observation that the clearance of MEHP was higher than that of DEHP. Non-linear increases in the AUCs of DEHP and MEHP indicated saturation in the formation as well as the elimination of MEHP. In mice the radioactivity was rapidly accumulated in the

kidney and liver after intravenous injection, with high concentrations in urine, bile, and intestinal contents. There was no evidence of retention in any tissues in the body. The secretion by the liver, via the bile into the intestine appears to be the major route. No enterohepatic circulation of DEHP was indicated since there was a persistently high concentration in the intestinal lumen.

Metabolism

The presence of significant amounts of MEHP and phthalic acid in circulation following exposure to DEHP via haemodialysis (Peck et al., 1978) means that the potential contribution of the biologically active de-esterified products to the long term toxicological effects of DEHP must be recognized. Judging from the plasma concentrations of DEHP and MEHP during and after transfusion, there was no gradual accumulation of these substances during the course of transfusions (Sjöberg et al., 1985d). Plasma concentrations of DEHP in infants declined at a faster rate than those of MEHP.

Excretion

Following intravenous administration to marmosets approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion) indicating a 2 to 1 ratio between the urinary and faecal routes of excretion (Rhodes et al., 1983). Around 28% of the dose remained in the lungs 7 days after administration, with minimal levels in other tissues. The cumulative ^{14}C excretion in urine of three Green monkeys was greater than 70% of the dose after 24 hours (Albro et al., 1981; Peck and Albro, 1982). The cumulative recovery of ^{14}C in the urine following an *i.v.* dose of DEHP in Rhesus monkeys was 61%, indicating that approximately 40% is either excreted by alternative routes (e.g. faeces) and/or retained (Wester et al., 1998).

A small proportion of the dose was excreted in marmosets following intra peritoneal administration (10% in the urine and 4% in the faeces) in a 2 to 1 ratio. Around 85% of the dose remained as unabsorbed DEHP in the peritoneal cavity with minimal amounts in the tissues (0.6%) (Rhodes et al., 1983 and Rhodes et al., 1986).

There are a few studies available indicating that the radioactivity from (^{14}C)DEHP can cross the placenta barrier and distribute into foetal tissues (Srivastava et al., 1989; Singh et al., 1975 and Lindgren et al., 1982).

In vitro studies

The in vitro studies have led to the proposition that no or very little unmetabolized DEHP will reach the systemic circulation after oral administration to less than massive amounts of this compound. Data on DEHP hydrolase activity in homogenates of intestinal tissues from rats, mice, hamsters, and guinea pigs suggested that orally ingested DEHP is hydrolysed to MEHP before absorption occurs (Albro and Thomas, 1973). DEHP was hydrolysed to MEHP by lipases from a variety of rat tissues with pancreas, liver, and intestinal mucosa containing the bulk of the DEHP hydrolase activity (Albro and Thomas, 1973). High plasma levels of MEHP and only small amounts of DEHP in peripheral blood corroborates this hypothesis (Sjöberg et al., 1985d). There was no difference in DEHP hydrolase activity between intestinal homogenates from young adult (200 g) and old (450 g) rats, but there was higher activity from male than from female rats, and from fed than from fasted rats (Albro and Thomas, 1973). Variation among species was not extreme, although different mouse strains showed considerable differences. Out of 15 tissue preparations, only the liver alkaline lipase preparation was able to further hydrolyse MEHP to phthalic acid.

4.1.2.1.7 Overall Conclusion

There are a limited number of studies on the toxicokinetic behaviour of DEHP in man. These reports concern exposure by the oral, intravenous and inhalatory routes, however, they contain limited information. A single study performed by the dermal route is not considered reliable. Toxicokinetic studies in animals have been performed by the oral, inhalatory, dermal and parenteral routes. The majority of these studies have, however, been performed by the oral route and in three different strains of rat. Several studies with non-human primates, including old world- and new world apes, and mice have been conducted. In addition, there are several studies including other species. By the parenteral routes has been most studied in non-human primates, but there are few studies by the dermal and inhalation routes. In addition, *in vitro* studies have also contributed to the understanding of the toxicokinetics.

The majority of the toxicokinetic studies as a whole are mainly research orientated and published in the open litreature. Dose levels greatly varied between studies. The quality and relevance of these studies is variable and it was necessary to examine the database as a whole to derive conclusions.

Generally, DEHP (probably in the form of MEHP), is rapidly absorbed from the gastrointestinal tract following oral administration. The extent of absorption in rats, non-human primates and humans is around 50% for doses up to about 200 mg/kg bw. At higher doses, it appears that absorption in non-human primates is dose-limited in contrast to rodents.

For humans, information is not, however, available concerning the dependency of oral uptake on dose. Also, the extent of oral absorption at doses which humans are expected to be exposed is not known. Absorption may be 100% at daily exposure levels. In addition, the oral absorption characteristics of human subpopulations e.g. age- and health dependent factors, is not known. Hence, for children it is considered appropriate to assume a 100% value for oral absorption. Because absorption via inhalation will comprise of respiratory and oral absorption, 100% bioavailability is also considered appropriate for children. Concerning dermal absorption, different bioavailability values were not selected for adults and children: This may be discussed. The selected bioavailability values are summarised:

Summary of exposure route dependent systemic bioavailability	
Human exposure route	**Human systemic bioavailability (%)**
Oral	
Adults	50
Infants/children	100
Inhalation	
Adults	75
Infants/children	100
Dermal (free DEHP and in products)	
Adults	5
Infants/children	5
Parential routes	
All subpopulations	100

For rats a threshold was reached (450 mg/kg bw) above which there was a steady increase in the amount of unhydrolysed DEHP reaching the liver. In contrast, an absorption threshold could not be determined in $C3B6F_1$ mice for doses up to 1,000 mg/kg bw. There are no data available concerning possible absorption threshold in humans. Since, human exposure to DEHP is via inhalation, dermal, parential routes, one would expect that intact DEHP could reach the liver.

Limited data on toxicokinetics, following inhalation or dermal exposure, indicate that DEHP can be absorbed through the lungs whereas absorption through the skin appears to be limited. Following intra peritoneal injection most of the administered dose remains in the peritoneal cavity.

Distribution studies have mostly monitored total radioactivity rather than particular substances, little is known about the tissue distribution of DEHP and its metabolites. The distribution studies indicate that the radioactivity from (^{14}C) DEHP is widely distributed in the body without evidence of accumulation in the tissues of rat. In a limited number of pigs and broiler hens, a lower degree of clearance of unlabelled DEHP is indicated. A comparative study of rats and marmosets showed similar distribution patterns in the two species (oral administration) whereas rats had higher tissue levels than marmosets. Thus, the difference in distribution between species is quantitative rather than qualitative.

The metabolism of DEHP involves several pathways and yields a variety of metabolites. The first step in the metabolism of DEHP is hydrolysis by lipases to MEHP and 2-EH. The lipases are found in all tissues surveyed but especially in the pancreas, indicating that most of DEHP hydrolysis occurs in the lumen of the small intestine, and that hydrolysis of absorbed intact DEHP can occur in the liver and blood. The absorption of DEHP in the intestine is increased following hydrolysis to MEHP.

MEHP is a relatively major component in urine of monkeys, guinea pigs and mice but was mostly not detected in rat urine. However, MEHP is present in plasma in all species tested.

The oxidative metabolism of MEHP in the liver begins with hydroxylation of the ethylhexyl side chain resulting in the formation of primary (ω-oxidation), and secondary ((ω-1)- and (ω-2)-oxidation) alcohols. These alcohols are then oxidized to diacids (ω-oxidation) or diketoacids ((ω-1)-oxidation), respectively. The diacids are apparently subject to α- or β-oxidation at the ethyl- or hexylchain, respectively, in mitochondria and peroxisomes to yield shorter diacids. This process does not extend beyond the branch point in the ethylhexyl-chain. Generally, excluding the first hydrolysis step to MEHP, the metabolism of phthalate esters is qualitatively unaffected by the route of administration.

The first hydrolysis step, the hydrolysis of DEHP to MEHP, is common to all investigated species. One species difference related to the metabolism of DEHP seem to be that oxidative metabolism of MEHP plays a dominant role in rats but not in non-human primates. Following oral exposure approximately 75% of urinary metabolites consisting of dicarboxylic acids (mainly metabolites V and I) derived by ω-oxidation of the ethylhexyl chain. However, after *i.v.* administration there seems to be a more equal distribution between ω- and (ω-1)-oxidation. The predominant metabolites in non-human primates are MEHP and metabolite IX (a secondary alcohol) derived by (ω-1)-oxidation. Metabolites V and I are only minor metabolites in non-human primates. Regarding DEHP-metabolism, mice exhibit metabolic profiles in common with both rats and primates. For instance, metabolite I derived from ß-oxidative metabolism of DEHP is a major metabolite (pathway) in rodents, and occurs at relatively high levels in mice, but not in non-human primates. Also high levels of MEHP and metabolites from (ω-1)-oxidation are the major metabolites in the urine from non-human primates and mice, but not from rats. The limited

human data indicate that human DEHP metabolism resembles that of other primates and of mice, with the exception of metabolite I in the latter case. However, regarding phase I metabolism of DEHP the species differences are quantitative rather than qualitative.

Glucuronidation is the major conjugation pathway identified in most species. In addition, ß-glucose conjugate was shown to be an alternative conjugation pathway in mice, but not in guinea pigs. Species differences have also been observed for glucuronidation since the fraction conjugated to glucuronic acid is absent in rats, low in hamsters, moderate in mice and guinea pigs. In primates, including humans, about 80% of the metabolites were glucuronides after intravenous administration, while for orally exposed humans about 65 and 99% of the metabolites, respectively, were glucuronides in two studies. The limited human data indicate that there are substantial inter individual differences e.g. polymorphism in the glucuronidation of some metabolites of DEHP, and of MEHP in particular. Mice that differ from rats in their ability to glucuronidate MEHP respond with the same types of toxic injury as do rats, indicating a possible partial independence of toxicity from the metabolic pathways in different species.

The elimination of DEHP largely depends on its metabolism and it might take 5-7 days to eliminate 80% of the radioactivity given as radiolabelled DEHP, either orally or intravenously. The half-life for DEHP and its metabolites was 3-5 days in the adipose tissue and 1-2 days in the liver. The elimination is most rapid in rats.

Many of the studies do not have complete recoveries, indicating that either biliary excretion and/or retention occur. Since many studies reveal that no significant retention was found in organs and tissues of labaratory test animals, biliary excretion appears to be an important excretion route. About 5-10% of the dose in rats was recovered from the bile in 24 hours after oral administration of DEHP, whereas about 24%, of the dose was recovered from the bile after intravenous administration of DEHP. In mice, biliary excretion seems to be one of the major route of exposure. In addition, metabolites are found in faeces from mice, rats and monkeys indicating that DEHP is absorbed, metabolised and excreted via the bile into the intestine. The extent of biliary excretion in humans is unknown. There are indications that resorption of radioactivity take place in the intestine of rats. Unfortunately, there is no data available on enterohepatic circulation of DEHP from other species than the rat, and the data on rats is very limited.

The radioactivity from (^{14}C) DEHP can cross the placenta barrier and distribute into foetal tissues. In addition, DEHP can be transferred through the milk from lactating rats to their pups. One study suggests that there are age-related differences since the extent of absorption Sjöberg et al. (1985c), and, hence, total systemic exposure to MEHP and its metabolites is higher in young rats than in old when DEHP is administered by gavage. Clearly higher blood levels were found in new-borns after blood transfusions, haemodialysis or treatment with platelet concentrates, compared to similarly exposed adults. Since the immature liver may have a lower metabolising capacity than that of older children and adults, infants and foetuses might be especially vulnerable to exposure of DEHP and MEHP.

Conclusion

The relative extent to which different metabolites are produced and excreted is very complex and may depend upon the species, the age of the animal, sex, inter-individual differences, state of health, nutrition state, prior exposure to DEHP, the amount of DEHP administered, the administration route etc. With the exception of non-existing glucuronidation in rats there is no reason to suspect that functionally equivalent pathways for the metabolism of DEHP differ significantly in higher species.

The available data on the toxicokinetics of DEHP cannot explain the species differences in the DEHP-induced toxic effects, and are consistently not adequate to support any conclusion on the relevance or irrelevance for humans of the DEHP-induced toxic effects in experimental animals.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Inhalation

In a study performed according to GLP principles, groups of 5 male and 5 female rats were exposed for 4 hours to clean air (control group) or DEHP (purity not specified) in concentrations of either 3.39, 6.82, or 10.62 mg/litre (3,390, 6,280, or 10,620 mg/m^3) (Hüls, 1981). The highest dose was considered the technical limit of aerosol generation for the test material. The control group and the lowest dose group were exposed on the same day. The mid-dose group and the highest dose group were exposed on different days. The exposure was nose-only. The rats were observed for clinical signs throughout the exposure period and for the first 4 hours after dosing. During the subsequent 14-day observation period the rats were inspected twice daily. Body weights were measured before exposure and with regular intervals during the observation period. A detailed macroscopic examination was performed on all animals at sacrifice at the end of the observation period. No animals died during or after the exposure. All treated animals showed a slightly unkempt appearance for 1-2 days after exposure, those in the highest dose group had a yellowish staining on their fur. This group also had a reduced body weight gain on the second day after exposure, which subsequently returned to the normal pattern. In all groups, dark red foci and patches were observed in the lungs at post mortem inspection. These findings were more frequent in the treated animals. In conclusion, the LC_{50} of DEHP via inhalation was in this study found to be in excess of 10,620 mg/m^3 for 4 hours.

Other acute inhalation studies exist. None of these studies are, however, useful for a risk assessment due to inappropriate design or poorly reported test methods and results.

Oral

The acute oral toxicity of DEHP has been investigated in several studies. A number of the available studies have been omitted from the risk assessment report because of limited quality of the studies, especially with respect to the identity of the test substance and the description of test methods.

Rats

Acute oral toxicity of DEHP in the rat has been estimated as a prerequisite to a carcinogenicity study by NTP (1982). Doses from 800 to 20,000 mg/kg bw of DEHP (99.5% pure) were administered in a single dose by gavage to groups consisting of 5 males and 5 females. The vehicle was corn oil. No deaths were observed during a 14-day observation period, giving an LD_{50} in excess of 20,000 mg/kg bw. No individual animal data are given.

In a study performed according to GLP principles and according to Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) standards, groups of 5 male and 5 female rats received either 5,000, 10,000, 20,000 or 40,000 mg/kg bw as single doses of undiluted DEHP (99.8%

pure) in various volumes (Nuodex, 1981a). Up to 40 ml/kg were administered. During the first days after dosing, all animals exhibited rough coat, decreased activity, and appeared wet on the posterior, later on these signs disappeared. The number of days with clinical symptoms were correlated to the dose. No deaths were observed. On day 14, all animals were sacrificed and subjected to gross necropsy. No abnormalities were observed. The LD_{50} was determined to > 40,000 mg/kg bw.

Mice

An acute oral toxicity study performed according to GLP principles (however, individual animal data for acute toxicity of single doses were not presented in the report) has been carried out as a preliminary study to a dominant lethal mutagenicity study (Nuodex, 1981b). The test substance was 99.7% pure DEHP. Initially, a range-finding study was performed, in which groups of 5 male mice were given oral doses of 100, 250, 500, 1,000, 2,500, 5,000, 7,500, or 9,860 mg/kg bw by gavage in corn oil. As no deaths occurred during a two-week observation period, a group of 10 male mice was given a single dose of 9,860 mg/kg by gavage ("LD_{50} study"). The animals were observed for 14 days. In the range-finding study, some animals exhibited slight depression and/or rough fur during the first day, but no deaths occurred. The LD_{50} was estimated to be greater than 9,860 mg/kg bw. In the LD_{50} study these findings were confirmed. All 10 mice survived and gained weight. Following treatment, the mice were depressed and had rough fur for 2 to 3 days; they also had a humped appearance several hours after treatment, lasting about one day. Necropsy at the end of the two-week observation period revealed no abnormalities. Thus, the acute oral LD_{50} in mice was > 9,860 mg/kg bw in this study.

Acute oral toxicity of DEHP in mice has also been estimated as a prerequisite to a carcinogenicity study by NTP (1982). Doses from 800 to 20,000 mg/kg of DEHP (99.5% pure) were administered as a single dose by gavage to groups consisting of 5 males and 5 females. The vehicle was corn oil. No deaths were observed during a 14-day observation period, thereby giving an LD_{50} value > 20,000 mg/kg bw. No individual animal data are given in the report.

The studies on rats and mice are summarised in **Table 4.29**.

Dermal

The acute dermal toxicity of DEHP has not been investigated in a study of guideline quality.

One study has been reported where rabbits were exposed dermally for 24 hours with doses up to 20 ml/kg (Shaffer et al., 1945). That amount killed 2 of 6 rabbits. The author concluded that the LD_{50} by skin absorption would be near 25 ml/kg (approximately 24,500 mg/kg bw). However, details of the experimental design and original results are not presented in the report.

No additional data relevant for the risk assessment have been identified.

Other routes

Acute toxicity studies using other routes of administration (intraperitoneal and intravenous) have not been performed according to a guideline or under GLP conditions.

LD_{50}-values ranging from 4,900 to 147,000 mg/kg bw have been reported for rats following intraperitoneal administration, and from 5,000 to > 128,000 mg/kg bw for mice.

Following intravenous administration, LD_{50}-values range from 250 to 2,080 mg/kg bw for rats and from 1,060 to 1,370 mg/kg bw for mice.

These studies are, however, not useful for the risk assessment due to inappropriate study design or poorly reported test methods and results.

4.1.2.2.2 Studies in humans

Shaffer et al. (1945) has presented a case report on two adult male subjects who had swallowed DEHP as single doses of 5 g and 10 g, respectively. No symptoms resulted from the 5 g dose while the ingestion of 10 g caused mild gastric disturbances and "moderate catharsis". No additional human data relevant for the risk assessment have been available.

4.1.2.2.3 Summary of single exposure studies

In a study of good quality, the toxicity of a single dose of DEHP via inhalation to rats was in excess of 10.62 mg/litre/4 hours.

The acute oral toxicity of DEHP has been studied in several experiments of good quality. The LD_{50}-value in rats is > 20,000 mg/kg bw and in mice > 10,000 mg/kg bw. Only one report on the acute oral toxicity in humans has been located; the ingestion of 5 g caused no adverse effects, while 10 g caused mild symptoms.

The acute dermal toxicity of DEHP has not been investigated in any study of good quality. Due to poor dermal absorption of DEHP, the acute dermal toxicity is expected to be low.

Following a single iv administration of DEHP in rats, effects were observed on the lungs including edema of the alveolar wall together with infiltration by leukocytes, hemorrage, and lethality (LD_{50}: 200 mg DEHP /kg; Schulz et al., 1975; Rubin and Chang, 1978). In another study, the DEHP metabolite MEHP was injected into five rats every minute (From 0-20 mg, the rate of injection was 0.46 mg per minute; at 20 mg, this rate was increased to 0.92 mg/minute; at 36.8 mg, the rate was increased to 1.9 mg/minute for 2 minutes, and to 3.7 mg/minute for the next 2 minutes, and then to 4.6 mg/minutes until the animals died. The total dose for rat 1: was 58 mg, rat 2: 72 mg, rat 3: 95mg, rat 4: 75 mg, and for rat 5: 95 mg.) through the femoral artery, and the blood pressure and heart rate were constantly recorded. For heart rate a LOAEL and NOAEL of 57 and 28.5 mg /kg bw, respectively, were derived; and for the drop in blood pressure a LOAEL and NOAEL of 214 and 157 mg /kg bw, respectively, were derived (Rock et al., 1987). These effects were not considered critical for the risk characteriasation due to the inadequacy of the reported data, lack of reproducibility as well as the severity and/or relevance of the observed effect. However, the mentioned effects, if substantiated, may be of concern for vulnerable populations such as newborns, elderly, and patients exposed to DEHP via medical equipments.

The data available on acute toxicity do not suggest a classification of DEHP according to EU criteria.

Table 4.29 Acute oral toxicity of DEHP in experimental animals

Species	Protocol	LD_{50} (mg/kg bw)	References
rat, F-344 5 rats/sex/group	gavage, corn oil 800-20,000 mg/kg bw comparable to guideline study	> 20,000	NTP (1982)
rat	methods not reported	> 20,000	BASF (1953)*
rat	methods not reported	> 9,800	BASF (1961)*
rat, F-344 5 rats/sex/group	gavage 5,000, 10,000, 20,000, or 40,000 mg/kg bw FIFRA standards Part 163, Title 40, GLP	> 40,000	Nuodex (1981a)
mouse	methods not reported	> 31,360	Lawrence et al. (1974)*
mouse, B6C3F1 5 mice/sex/group	gavage, corn oil 1,250-20,000 mg/kg bw comparable to guideline study	> 20,000	NTP (1982)
mouse	5,000 or 10,000 mg/kg bw methods not reported	> 10,000	BASF (1941)*
mouse, ICR/SIM 5 or 10 males/group	gavage, corn oil 100, 250, 500, 1,000, 2,500, 5,000, 7,500, or 9,860 mg/kg bw GLP	> 9,860	Nuodex (1981b)

* Study of less importance for the risk assessment

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin irritation

The acute dermal irritative and corrosive properties of DEHP have been investigated in a study conforming with OECD guideline 404 (BASF, 1986a). Information about the identity of the test substance is not enclosed with the test report. The test substance is referred to by the name "Palatinol AH", Batch. No. 155 H, Sept. 85. In BASF (1961), the identity of Palatinol AH is given as DEHP. Three White Vienna rabbits were clipped free of hair on an area of skin on the upper third of the back or flanks. A 2.5 cm · 2.5 cm gauze patch wetted with the undiluted test substance was applied to the skin for 4 hours. The remaining test substance was removed from the skin. Untreated skin sites of the same animal served as negative control. Observations of degree of erythema and oedema were made according to the guideline scoring system 30-60 minutes after removal of the test patch, and at 24, 48, and 72 hours after the beginning of application. All rabbits scored zero on both parameters at all observation points.

In another study, also performed in accordance with OECD guideline 404, skin irritation of DEHP (> 99% pure) was studied (Hüls, 1987a). The hair was clipped from the dorsal and lateral part of the trunk of three male Little White Russian rabbits. The undiluted test substance

(0.5 cm^2) was applied to a skin area of 6 cm^2 and covered with a patch. After 4 hours, the patch was removed and the skin gently rinsed with warm water. The skin reactions were evaluated after 1, 24, 48, and 72 hours, and after 6 and 8 days, using the grading system of the OECD guideline. At the 1-hour observation, all rabbits showed very slight erythema, and one rabbit also a very slight oedema. At 24 hours, the reaction of one rabbit had progressed to a well-defined erythema. At 48 hours, all rabbits had very slight erythema which at 72 hours was accompanied by a dry appearance of the skin. At 6 days, the skin surface appeared scaly, and at 8 days the skin was free from reaction. It was concluded that DEHP was slightly irritating to the skin.

In a skin irritation study performed according to GLP principles and conforming with the U.S. Food and Drug Administration (FDA) recommended method, 3 male and 3 female New Zealand White rabbits were used (Hüls, 1981). The skin of the back was clipped free of hair and 2 of the 4 patch test areas were abraded. One square inch chromatography paper patches were wetted with the undiluted test substance (DEHP), or with 10% aqueous sodium lauryl sulphate (as positive control substance). Patches were applied to both intact and abraded skin and left in position for 24 hours, thereafter the skin was cleansed. The test sites were scored immediately (24-hour reading) and 48 hours later (72-hour reading). Reactions were evaluated according to the FDA recommended scoring system. DEHP caused mild to moderate reactions at 24 hours. At the 72-hour reading, no treated sites showed any response to treatment.

Other skin irritation studies have been carried out. As the methods and results are poorly reported and do not add any further information, these studies have been omitted in the risk assessment report.

Eye irritation

In an acute eye irritation study performed in accordance with OECD guideline 405, the test substance was mentioned as “Palatinol AH”, Batch. No. 155 H, Sept. 85 (BASF, 1986a). Palatinol AH is identified as DEHP in a BASF study from 1961. Three adult White Vienna rabbits received a single application of 0.1 ml undiluted test substance to the conjunctival sac of the right eye. The left, untreated eye served as negative control for each animal. The test substance was not washed out. The animals were observed 1, 24, 48, and 72 hours after application. The observations were graded according to the guideline scoring system. The average score value for 24, 48 and 72 hours was 0.1 for conjunctiva reddness and 0.0 for cornea opacity, irsis and swollen conjunctiva.The results do not fulfill the EU criteria for eye irritation.

In another study also performed in accordance with OECD guideline 405, eye irritation of DEHP (>99% pure) was studied (Hüls, 1987b). DEHP in a volume of 0.1 ml was installed in the right eye of three male Little White Russian rabbits. The left eye served as control. The animals were evaluated after 1, 24, 48, and 72 hours, and after 6 days of application, using the grading system of the OECD guideline. At 1 hour, the conjunctivae of all three rabbits showed mild redness and one rabbit showed mild discharge. No conjunctival reactions were observed at the later observation times. All observations for chemosis, corneal opacity and lesions of the iris were negative.

In a study performed according to GLP principles and conforming with the U.S. Food and Drug Administration (FDA) recommended method, DEHP in a volume of 0.1 ml was introduced into the right eye of 3 male and 3 female New Zealand White rabbits (Hüls, 1981). The eyes were examined at 1, 24, 48, and 72 hours, and at 7 days, and the reactions were scored according to the FDA recommended scoring system. No reactions were found in the cornea or iris at any point of time. The conjunctivae of 5 eyes exhibited mild redness at 1 hour, while one eye showed very

mild redness. At 24 hours, mild redness persisted in 3 eyes, while the remainder had no redness. No redness was observed at 72 hours or 7 days.

Other studies of eye irritation have been performed. As the methods and results are poorly reported and do not add further information, these studies have been omitted in the risk assessment report.

Respiratory tract irritation

No studies specifically addressing this issue have been found.

In a study of acute toxicity by inhalation, groups of rats were exposed to DEHP in concentrations of 3.39, 6.82 or 10.62 mg/litre for 4 hours (Hüls, 1981). The respiratory tract was subjected to detailed macroscopic examination in all animals and revealed dark red foci and patches in the lungs. These foci and patches were observed more frequently in the treated animals (present in 19 out of 30 rats exposed to DEHP) than in the controls (present in 2 out of 10 rats exposed to clean air). The lung-to-body weight ratios of all treated groups were similar to the ratios obtained in the control group.

4.1.2.3.2 Studies in humans

Skin

Shaffer et al. (1945) found no erythema or any other reaction resulting at any time following application of undiluted DEHP as patch tests in 23 human subjects. The substance was applied on the back, left in contact for 7 days and reapplied on the same spots 10 days later.

4.1.2.3.3 Summary of irritation

DEHP has been tested for skin irritation in three well reported animal studies, conducted in accordance with guidelines and the principles of GLP. In two studies, DEHP was found slightly irritating to the skin. The irritation was not severe enough to warrant a classification for skin irritation according to the EU criteria.

Three well reported studies for eye irritation have been presented. The studies followed existing guidelines and were performed in accordance with GLP. The results of the studies show that DEHP is slightly irritating to the eye. The irritation was transient and not severe enough to warrant a classification for eye irritation according to the EU criteria.

Irritation to the respiratory tract cannot be assessed. The results of one study designed to give information about acute toxicity by inhalation suggest a potential for DEHP to induce lung lesions. However, the nature of the lesions was not investigated microscopically, nor was the dose-response relationship explored.

The available human data are not adequate for the risk assessment.

4.1.2.4 Corrosivity

See Section 4.1.2.3.

4.1.2.5 Sensitisation

According to the TGD, the adjuvant type test (the guinea pig maximisation test) is the preferred method for determination of sensitisation. The Buehler test is, however, also acceptable.

4.1.2.5.1 Studies in animals

Skin sensitisation

One Magnusson-Kligman guinea pig maximization test has been performed, in which female albino Dunkin-Hartley guinea pigs were used (Hüls, 1981). The maximisation test comprised two procedures. The induction procedure consisted of an intradermal injection of the test material into the skin of the shoulder region followed by a topical application 7 days later. The intradermal injection (actually 3 injections) consisted of 0.1 ml Freund's adjuvant alone, 0.1 ml 10% DEHP in paraffin oil, and 0.05 ml 10% DEHP in paraffin oil emulsified with 0.05 ml Freund's adjuvant. The control group received 2 injections of Freund's adjuvant only. For the topical application, a 2 · 2 cm patch of filter paper was wetted with 50% DEHP in paraffin oil and applied for 48 hours to the pre-treated area. The control group was not subjected to topical application. The challenge procedure which consisted of a topical application was carried out 14 days after the completion of the induction period. In preliminary experiments, a solution of 50% DEHP was determined to be non-irritant; higher concentrations were not tested. A 2 · 2 cm patch wetted with 50% DEHP was applied to a challenge site (on the right flank) of all animals for 24 hours. The degree of response was assessed 24 hours after removal of the challenge patch and rated. Any animal showing erythema at the challenge site was considered to have shown a positive response. DEHP was unequivocally not sensitising in the guinea pig maximization test; there were no positive responses.

A Buehler test has been performed according to Annex V Methods (Annex of Directive 92/69/EEC, Part B) and GLP principles (Exxon, 1994). The test substance was DEHP of unspecified purity (Jayflex DEHP). Forty female nulliparous and non-pregnant albino Dunkin/Hartley albino guinea pigs were allocated either to the control group (20 animals) or to the test group (20 animals). The strain is documented sensitive to the sensitisation caused by formalin. A preliminary study was carried out in 4 additional animals to identify possible irritancy of a range of dilutions of the test substance (40, 60, 80, and 100%, using Alembicol D as vehicle). The animals were observed at 0, 24, and 48 hours. No irritancy was shown in the preliminary study, and the undiluted test substance could therefore be used for the main study. The procedure may be considered in two parts, induction and challenge.

Induction: the skin on the left shoulder region of the guinea pigs was clipped free of hair. Three induction applications were made on days 1, 8, and 15. Each time, a gauze patch saturated with the test substance was maintained in contact with the skin for 6 hours, after which the patches were removed. The control animals were treated similarly with the exception that the test substance was omitted. The dermal reactions were observed immediately and 24 hours after each induction application.

Challenge: two weeks after the last induction application all animals were challenged using a new, clipped skin area on the right flank. The challenge application was identical to the induction. The challenge sites were evaluated 24, 48, and 72 hours after removal of the patches using a scoring system.

The results of this study clearly show no sensitising properties of the test substance, as no dermal reactions were seen in any of the test or control animals at any point of time during the induction and challenge phase. There were no clinical signs of toxicity and no effects on body weight.

Other relevant studies have not been available.

Respiratory tract sensitisation

An in vitro study investigating the bronchial hyperresponsiveness to DEHP in rat tracheal tissue has been reported (Doelman et al., 1990). Methacholine dose response curves of rat tracheal tissue were not influenced by DEHP after incubation for 30 minutes. Incubation with 10^{-4} M MEHP induced a significant dose-dependent increase in -log EC_{50} for methacholine in rat tracheal tissue compared to control. MEHP-incubation also resulted in a decrease in maximal effect for methacholine but only at relatively high concentrations. The effects seemed to be reversible. After a 60-minute washing period, -logEC_{50} and maximal response for methacholine returned to normal. The authors concluded that only continous exposure to DEHP might cause bronchial hyperresponsiveness.

4.1.2.5.2 Studies in humans

A case study on occupational asthma due to DEHP has been described in worker at a PVC-processing plant (Brunetti and Moscato, 1984). The orignal study (in Italian) was briefly reported in a review (IPCS, 1992) without giving any experimental data. Therefore, it is impossible to evaluate the relevance of this study.

The role of interior surface materials in the home in the development of bronchial obstruction (as an indicator for development of asthma) during the first 2 years of life was assessed in a case-control study based on the Oslo Birth cohort (246 case subject and 246 age mached controls) (Jaakkola et al 1999).The results showed that the risk of bronchial obstruction was greater in the presence of PVC in the floors, compared with the reference category of wood or parquet flooring and painted walls and ceiling. On the other hand, the risk of bronchial obstruction was not related to PVC wall materials. It was also reported that the risk of bronchial obstruction increased in relation to the amount of plasticizer-emitting materials in the home. In another study published by the same group (Øie et al., 1997), the exposure to plasticizers from sedimented dust samples and suspended particulate matter samples collected from different parts of dwellings selected from a reported “Oslo Birth cohort Study” was assessed. Different amounts of different phthalate esters were detected in these samples: DEHP was predominant in both the sedimented dust samples (69% of total phthalate) and the suspended particulate matter samples (52% of total phthalate).

In conclusion, the presented studies indicate that the risk of bronchial obstruction and asthma may increase in the presence of DEHP and other plasticizer in PVC products among a number of other chemicals and factors in the indoor environment. However, there is no sufficient evidence supporting a causial association between exposure to DEHP and induction of asthma.

Shaffer et al. (1945) found no erythema or any other reaction at any time following application of undiluted DEHP. Twenty-three human subjects had been subjected to patch tests upon the back where DEHP was left in contact for 7 days and reapplied on the same spots 10 days later.

4.1.2.5.3 Summary of sensitisation

DEHP has been tested according to the Magnusson-Kligman Guinea Pig Maximization test as well as the Buehler test with negative results indicating that DEHP is no skin sensitiser in animals.

Limited *in vitro* data indicate that DEHP due to the formation of MEHP might provoke bronchial hyperresponsiveness.

The available human data are not adequate for a risk assessment.

The available sensitisation data do not suggest a classification of DEHP according to EU criteria.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in humans

A case report suggests that inhalation of DEHP may induce toxic damage of the lungs in the preterm infant. In preterm infants, artificially ventilated with PVC respiratory tubes, unusual lung disorders resembling those observed in hyaline membrane disease were observed during the fourth week of life in two infants (Roth et al., 1988). In a third infant, who died two weeks after birth, DEHP was detected in the lung tissue but not in the liver tissue. The estimated inhalative exposure in the three infants ranged between 1μg/hour – 4,200 μg/hour. DEHP, but not MEHP, could be demonstrated in urine samples. The authors assumed that these findings were causally related to the exposure to high doses of DEHP released from the PVC tubes.

A morbidity study was carried out on a group of 97 men and 4 women employed in a German plant producing DEHP (Thiess et al., 1978b). The average exposure period was 12 years (4 months to 35 years). Background levels were generally low (0.001-0.004 ppm ≈ 0.016-0.064 mg/m^3) with higher levels up to 0.01 ppm (0.16 mg/m^3) in the vicinity of the chemical reactor. Blood lipids, serum activities of liver enzymes, and routine haematological tests were normal, and no excess of any pathological condition was found. All 58 children fathered by the exposed men were normal. Due to the low exposure levels and the lack of a referent group, this study is considered inadequate with respect to the risk assessment.

A mortality study of 221 workers exposed to DEHP in the plant was also conducted. Eight deaths occurred in the cohort compared with expected values of 15.9 and 17.0 for city and country, respectively (Thiess et al., 1978c). This study is considered inadequate with respect to the risk assessment due to small cohort size, short follow-up, and low exposure levels.

Three epidemiological studies on neurological symptoms in workers exposed to phthalate esters, including DEHP, by inhalation are available. However, due to several limitations including lack of an appropriate referent group, small size of the exposed population, inadequate documentation, and mixed exposure to other substances than DEHP, these studies are considered inadequate with respect to risk assessment.

A morbidity study was conducted in the USSR of 147 workers at a PVC-processing plant (Milkow Milkov et al., 1973). The workers were exposed to a mixture of phthalates, including DEHP as a minor constituent. Tricresyl phosphate (a neurotoxin) was a component of the incombustible materials produced in 10-20% of machines assigned to various workers. The total phthalate air concentrations recorded varied between 1.7 and 66 mg/m^3. No referent group was

included in the study. Frequent complaints of ill-effects were made by those exposed to phthalates. A high incidence of pain in the upper and lower extremities was reported in 57% of those employed for 6-10 years and 82% of those employed for more than 10 years. Polyneuropathy was evident in 47 workers (32%); the incidence increased with length of employment. Another 22 workers (15%) were said to have functional disorders (not specified) of the nervous system. Vestibular abnormalities were evident in 63 workers (78%) of 81 workers specifically examined.

In a cross-sectional study, symptoms and signs of polyneuropathy were reported in 12 out of 23 workers at a plant for phthalate production in Italy (Gilioli et al., 1978). The workers were exposed to a mixture of phthalates, including DEHP, but also, to a lesser degree, to the corresponding alcohols and to phthalic anhydride. Total phthalate air concentrations recorded varied between 1 and 60 mg/m^3. No referent group was included in the study. The authors concluded that no definite conclusion could be drawn from the study because of the limited number of workers examined. The study is reported in Italian with an abstract in English.

In a study involving a Swedish PVC-processing factory, 54 male workers were examined for peripheral nervous system symptoms and clinical signs. The workers were exposed mainly to DEHP, di-isodecyl phthalate, and butylbenzyl phthalate (Nielsen et al., 1985). They were divided into three groups of approximately equal size and with mean phthalate exposures of 0.1, 0.2, or 0.7 mg/m^3. Some workers displayed various peripheral nervous system symptoms and signs, but these were not related to the level of exposure. None of the workers reported symptoms indicating work-related obstructive lung disease. Neither did conventional lung function test results show any association with exposure levels. Some biochemical parameters (haemoglobin, α-1-antitrypsin, and immunoglobulin A) showed exposure-related associations.

There are no data available concerning peroxisome proliferation in humans exposed to DEHP. Data from studies of other peroxisome proliferators (hypo-lipidemic drugs) do not indicate that humans are sensitive to peroxisome proliferation.

Conclusion: the available studies in humans is inadequate for risk assessment.

4.1.2.6.2 Studies in animals

A few inhalation studies in experimental animals are available and the only study available following dermal exposure to DEHP is inadequate for risk assessment.

A large number of studies have investigated the toxicity of DEHP following repeated oral administration to experimental animals, mainly rats. The most pronounced effects included, effects on the liver (hepatomegaly, peroxisome proliferation, and replicative DNA synthesis), testes (tubular atrophy), effects on the kidneys and cardiopulmonary tissues andhypo-lipidemic effects (decreased plasma levels of cholesterol and triglyceride). Other, less pronounced, effects have also been observed, e.g. reduced body weight and body weight gain, and alterations in clinico-chemical parameters. The oral studies in rats and mice are summarised in **Table 4.30** with dietary intakes converted from ppm and percent into mg/kg bw.

4.1.2.6.3 Short-term repeated dose studies (up to 28-days exposure)

Inhalation

Rats

Wistar rats (10 males and females per group in the main dose group; 2 males and females per group in satellite group I, 15 males and 2-5 females per group in satellite group II; an equal number of control rats in each group; 9 weeks old at the beginning of exposure) were exposed in head-nose inhalation systems to DEHP (99.7% pure) aerosols of respirable particle size (mass median aerodynamic diameter < 1.2 ± 2.9-9.5 μm) or air (controls) (BASF, 1990 and Klimisch et al., 1992). Exposure duration was 6 hours per day, 5 days per week for 4 weeks at 0, 0.01, 0.05, or 1.0 mg/litre (0, 10, 50, or 1,000 mg/m^3). The animals of the main dose group were sacrificed at the end of the exposure period. Before sacrifice, male rats from satellite group II had a recovery period of 2 or 6 weeks after termination of exposure. Livers of animals from satellite groups I and II were examined by light and electron microscopy. No animals died during the study. Clinical examination and blood chemistry parameters did not reveal treatment-related effects. Body weights of treated rats and controls were similar. In the highest dose group, a significant increase in relative lung weights was seen in male rats. This was accompanied by foam cell proliferation and thickening of the alveolar septi. Absolute liver weights (females) and relative liver weights (both sexes) were slightly but significantly increased but there were no corresponding histological findings. All these effects were reversible within the post-exposure observation period. No testicular toxicity was detected histologically. Electron microscopical examination of liver samples from all three concentration groups and controls at the end of exposure and after the post-exposure period did not reveal clear ultrastructural changes in hepatocytes that could be attributed to the exposure or to peroxisome proliferation. The NOAEL in this study is 50 mg/m^3. It should be noted that the conclusions of this study are not always based on described values. For example, no evidence supporting the reversibility of the liver and lung effects is presented; also the histopathological results and information on the absolute and relative weights of the testis is lacking. Furthermore the reliability of this study is questioned since effects on peroxisome proliferation were not observed in contrast with a similar study conducted earlier by BASF (Merkle et al., 1988). In that report reference is made to a range-finding study in which "exposure-related" peroxisome proliferation was observed in dams from 200 to 1,000 mg/m^3. Therefore, this study is not considered reliable for risk characterisation.

In a range-finding teratogenicity study (BASF, 1986b as cited by Merkle et al., 1988), pregnant rats were exposed in a head/nose regimen to DEHP aerosol concentrations of 0, 0.2, 0.5 or 1.0 mg/litre (0, 200, 500 or 1,000 mg/m^3; MMAD < 1.2 ± 5.8 –16.8) (purity: the maximum level that was technically achievable) from gestation day 6 through 15. The animals were sacrificed on day 16. Electron microscopic evaluation (two rats per dose group) revealed peroxisome proliferation with an increasing trend at all dose levels, from slight at the control level to very marked at the highest exposure level. In each of the 200 and 500 mg/m^3 dose groups, one animal showed moderate and one animal very marked peroxisome proliferation. No effect on body weight gain or food consumption was observed in the highest dose group. This study is inadequate for risk assessment as no original data are available.

Peroral

Rats

Fischer 344 rats (5 animals/sex/group) were given 0.67% DEHP (purity not specified) in the diet for 28 days (Hodgson, 1987). No effects were observed on body weight gain and food intake. Relative liver weights were significantly increased in dosed rats compared to controls. A moderate increase in the number of peroxisomes and induction of three enzymes (PCoA, catalase, and CAT) indicative of peroxisome proliferation was observed.

In a following study, the effects of feeding either DEHP or 2-EH (purity: not specified) were compared. Fischer 344 rats were given 100, 320, or 950 mg/kg bw/day of 2-EH or 700 mg/kg bw/day of DEHP by gavage (vehicle not specified) for 21 days. A control group was included in the study. No further details of study design are given. A significant decrease in body weight gain was observed in the high-dose 2-EH-group but not in the other dosed groups. Relative liver weights were significantly increased in the high-dose 2-EH-group and in the DEHP-group when compared to the control group. The number of peroxisomes was slightly and moderately increased in the high-dose 2-EH-group and in the DEHP-group, respectively. Induction of PCoA, LAH-11, and LAH-12 was observed in the high-dose 2-EH-group and in the DEHP-group. At 950 mg/kg bw/day, 2-EH caused approximately the same increase in enzymatic activity as did 700 mg/kg bw/day of DEHP. Male Wistar rats (4 rats per dose group, 6 control rats) were fed a diet containing 0 or 2% DEHP (> 99.5% pure) for 3, 10, or 21 days (Mann et al., 1985). Body weights were significantly decreased after 10 and 21 days of treatment. Relative liver weights were increased in all dosed rats; electron microscopic examination showed significantly increased peroxisome proliferation, changes in mitochondria, and proliferation of the smooth endoplasmatic reticulum already after 3 days of treatment. The activities of peroxisomal enzymes (PCoA, α-GD, and catalase) were significantly increased.

In a study comparable to a guideline study and performed according to GLP principles, Fischer 344 rats (5 animals/sex/group) were fed diets containing 0.2, 0.67 or 2.0% DEHP (99.8% pure) for 28 days (Nuodex, 1981c). The average weekly body weights were significantly reduced in high dose males from day 14 of exposure and in high dose females throughout the study. The mean absolute and relative liver weights were significantly and dose-dependently increased at all dose levels in both sexes. The livers of all rats in the highest dose group were enlarged. No microscopically lesions were observed in adrenals, liver, spleen, heart, testes, ovaries, kidney, or brain of treated rats. Total lipid levels were significantly and dose-dependently reduced at all dose levels in both sexes. The activities of hepatic catalase and CAT were significantly increased in all dose groups.

In an other study comparable to a guideline study and performed according to GLP principles, Alderley Park rats (10 animals/sex) were given 2,000 mg/kg bw/day of DEHP (99.7% pure) by gavage in corn oil for 14 days (ICI, 1982 and Rhodes et al., 1986). The control group was given the vehicle. The body weight gain was significantly reduced in males but not in females. Slight signs of systemic toxicity were observed in treated rats of both sexes. Plasma cholesterol and triglyceride levels were significantly reduced in male rats but not in females. Absolute and relative liver weights (both sexes) and kidney weights (females) were significantly increased. Testis weights were significantly reduced in 3 male rats and testicular tubular atrophy was observed in 4 male rats. Brain weights were unaffected. Electron microscopy of livers revealed marked peroxisome proliferation (an 8-fold increase in males and 5-6-fold increase in females). Both number and size of peroxisomes were increased. Disorganisation of rough endoplasmic reticulum, mild proliferation of smooth endoplasmic reticulum, and increased number of

lysosomes and mitochondria were observed in a number of rats. A significantly increased activity in the peroxisomal enzymes α-GD and PCoA and an increased activity in catalase (males only, not significant) was observed. Peroxisome proliferation was also observed in the proximale tubule of the kidney (2-fold increase in both sexes).

In a study comparable to a guideline study and performed according to GLP principles, male Fischer 344 rats (8 animals) were fed 2% DEHP (100% pure) in the diet for one week (Exxon, 1982 a,b). A slight body weight loss was observed but was not significant when compared to the control group. Food consumption was significantly reduced. No changes in haematological parameters were observed. The absolute and relative liver and kidney weights were significantly increased compared to controls. No compound-related microscopic changes were observed in testes, liver, or kidneys. Cholesterol and triglyceride levels were significantly reduced.

In a study comparable to a guideline study and performed according to GLP principles, Fischer 344 rats (5 animals/sex/group) were fed 0, 0.01, 0.1, 0.6, 1.2, or 2.5% DEHP (99.9% pure; corresponding to 0, 11, 105, 667 1,224, or 2,101 mg/kg bw in males and 0, 12, 109, 643, 1,197, or 1,892 mg/kg bw/day in females) in the diet for 21 days (CMA, 1984b; Barber et al., 1987). In the highest dose group, rats of both sexes lost weight during the first week of the study and body weights were significantly reduced compared to controls from day 3 of the study. Animals given 1.2% gained less weight than the controls during the first three days of treatment, although the differences were not significant. Food intake was significantly decreased in the highest dose group (both sexes) and in females from the 1.2% dose-group. Absolute and relative **liver** weights were significantly increased from 0.6%. Histological examination showed a reduction in cytoplasmic basophilia in livers from male rats given 0.6% and more and in female rats given 1.2% and more. Relative kidney weights were significantly increased in the highest dose group; no histological abnormalities were, however, observed. Testis weights were significantly reduced in male rats in the highest dose group and moderate to severe testicular atrophy were noted. In male rats, serum triglyceride levels were significantly increased at 0.01% but significantly reduced from 0.6%. In female rats, serum triglycerides were significantly increased from 1.2%. There was no dose-related reduction in serum cholesterol levels. Electron microscopy revealed a dose-related increase in numbers of peroxisomes from 0.1% in males and from 0.6% in females. At dietary levels of 0.6% and above, the size range of the peroxisomes was also increased and there were changes in peroxisomal morphology. PCoA showed a dose-related increase at dietary levels of 0.6% and above. LAH-11 and LAH-12 were increased in males from 0.1% and more and in females from 1.2% and more. No NOAEL can be derived from this study. The LOAEL is 0.01% in the diet (corresponding to 11 mg/kg per day in males and 12 mg/kg per day in females).

In an other study comparable to a guideline study and performed according to GLP principles, Fischer 344 rats (4-5 animals/sex/group) were fed 0, 0.1, 0.6 or 1.2% DEHP (purity > 99%) (0, 80, 480, or 960 mg/kg/day) in the diet for one or three weeks (CMA, 1982c). A post observation period of 2 weeks followed. No effects on growth and feed consumption were noted. Dose-dependent increases (significant) in liver weights were seen from week 1 and throughout the study. Following the recovery period, liver weights decreased considerably but remained significantly higher in mid- and high-dose males and in high-dose females. Hepatocellular hypertrophy was seen in high-dose males and in 3 of 5 mid-dose males after 1 and 3 weeks, and in high-dose females after 3 weeks. After the recovery period, this effect was seen only in high-dose males. An increased number of peroxisomes was observed in high-dose males but was less marked in females. Catalase activities showed moderate increases in high-dose animals at 1 week of treatment. After 3 weeks, catalase levels remained elevated in high-dose males, and to a lesser extent in the females. Following the recovery period, catalase activities were similar to

controls. Activities of CAT exhibited marked dose-dependent and significant increases after 1 week, decreased substantially during the recovery period but remained significantly higher in mid- and high-dose animals than in controls. Dose-related moderate increases in kidney weights were observed in rats of the two highest dose groups. Other organ weights (brain, spleen, testes, and thyroids) were not significantly altered. Serum triglycerides were significantly reduced at all dietary levels after 1 and 3 weeks but normalised during the recovery period. Serum cholesterol levels were significantly reduced after one week in mid- and high-dose females and to a lesser extent in males. After three weeks, cholesterol levels remained lower in females but were normal in males. Cholesterol levels were normal in all treated rats following the recovery period. No NOAEL can be derived from this study. The LOAEL is 0.1% in the diet (corresponding to about 80 mg/kg per day).

In a study performed according to GLP principles, DEHP (99.9% pure) was fed to groups of 5 male Fischer 344 rats at dietary levels of 0.02, 0.05, 0.1, 0.5, 1.0, or 2.5% (24, 52, 115, 559, 1,093, or 2,496 mg/kg bw/day) for 28 days (BIBRA, 1990). The control group (10 male rats) received basic diet. A significant reduction in body weight and a reduced food intake was observed after 7 days of treatment in high-dose rats and was persistent throughout the study. Absolute liver weights were significantly increased from 0.5% while relative liver weights were significantly increased at all dose levels. Hepatic PCoA was significantly increased from 0.1% in the diet. Testis weights were significantly reduced at 2.5% in the diet; marked testicular atrophy was observed histologically. This study identifies no NOAEL; the LOAEL corresponds to the lowest dose administered, that is 0.02% in the diet (24 mg/kg/day).

Male Sprague-Dawley rats (5 animals per group) were given 25, 100, 250 or 1,000 mg/kg bw/day of DEHP (> 99% pure) by gavage in corn oil for 14 days (Lake et al., 1984b). Control rats (5 animals) received the vehicle. Relative liver weights were significantly and dose-dependently increased in the three highest dose groups. A marked dose-dependent increase in the activities of PCoA and CAT was also observed.

Male Fischer 344 rats (5 animals per group) were fed 0, 100, 1,000, 6,000, 12,000 or 25,000 ppm (0, 11, 105, 667, 1,223, or 2,100 mg/kg bw/day) of DEHP (99.8% pure) in the diet for 21 days (Short et al., 1987). The relative liver weight and PCoA oxidation in liver homogenates were significantly increased from 6,000 ppm. LAH-11 and LAH-12 hydroxylation were significantly increased from 1,000 ppm. Peroxisome proliferation was examined by electron microscopy and evaluated as moderate to very marked in the three highest dose groups.

In a study designed to reveal a NOAEL for peroxisome proliferation, male Wistar rats (6 animals per group) were fed 0, 60, 200, 600, 2,000 or 6,000 mg/kg of DEHP (> 98.4% pure) (0, 5, 18, 52, 182, or 549 mg/kg bw/day) in the diet for 2 or 4 weeks (RIVM, 1992). Peroxisome proliferation was evaluated by morphometric analysis (light and electron microscopy) and by measurement of the activity of peroxisomal associated enzymes (PCoA, ECoA, catalase, CAT, LAH-11, and LAH-12). There were no significant differences in body weights between control animals and treated groups. The liver weights of animals in the two highest dose groups were significantly increased in a dose-related manner compared to the control group following 2 or 4 weeks of treatment. The morphometric analysis revealed a significant increase in volume density and number of peroxisomes in animals given 200 mg/kg DEHP or more in the diet for two weeks. For all enzymes, a dose-response relationship was observed. The NOAEL for induction of LAH-11, LAH-12, and ECoA was 200 mg/kg, for PCoA 600 mg/kg, and for catalase 2,000 mg/kg. The activity of CAT (the most sensitive parameter in the study) was significantly increased in all treated groups. An overall NOAEL for peroxisome proliferation was established as 60 mg/kg DEHP (5 mg/kg bw/day) in the diet. The results for CAT were not taken into account in the establishment of the NOAEL as this enzyme is not specific for peroxisome proliferation.

In an attempt to relate the chemical structure to biological activity, the hepatic effects of DEHP and related compounds on hepatic peroxisome proliferation and peroxisomal enzymes have been studied (Moody and Reddy, 1978). Male Fischer 344 rats (4-5 animals per group) were given 2% of DEHP, 2-EH, 2-ethylhexyl aldehyde, and 2-ethylhexanoic acid in the diet for 3 weeks. Hepatic peroxisome proliferation in association with an increase in liver size, increase of hepatic activities of the peroxisome-associated enzymes catalase and CAT, and hypo-lipidemia were observed in all treated groups. The changes induced by 2-EH and 2-ethylhexanoic acid were comparable to those induced by DEHP whereas those of 2-ethylhexyl aldehyde occurred to a lower extent.

The induction and deduction of peroxisomal enzymes by DEHP was studied in male Wistar rats (3 animals per group) (Miyazawa et al., 1980). One group of rats was administered 2% DEHP (purity not specified) in the diet for 4 weeks. Another group was administered DEHP for 14 days followed by the control diet for 14 days. The liver weight increased during treatment with DEHP and decreased in the withdrawal period. The activities of enzymes of peroxisomal β-oxidation and of catalase were markedly increased by DEHP. The time required to reach half-way to the maximal induction for enzymes of peroxisomal β-oxidation was 5 to 7 days, whereas that for catalase was 3 days. On the withdrawal of DEHP, activities of the β-oxidation system and of catalase decreased to the control levels with a half-life of 2 to 3 days.

Female Sprague-Dawley rats were given 5 oral doses of 2 000 mg/kg bw/day of DEHP (> 99% pure) in corn oil by gavage on days 2-6, 6-10 or 14-18 of lactation (Dostal et al., 1987a) (see also Section 4.1.2.10.5). The rats were sacrificed 24 hours after the last dose. The body weights of **lactating rats** and of their suckling pups were significantly reduced in all treatment intervals. Food consumption was reduced in the mothers dosed on days 14-18. Relative liver weights were increased in the lactating dams at all three stages of lactation but not in the suckling pups. The hepatic peroxisomal enzyme activities (PCoA and CAT) were increased by 5- to 8-fold in treated dams at all three stages of lactation. Two-fold increases in these enzyme activities were also observed in pups suckling the treated dams. Also hypo-lipidemia was observed in treated lactating rats at all three stages of lactation. Plasma cholesterol and triglyceride concentrations were decreased by 30-50%.

In a following experiment, female Sprague-Dawley rats were given 3 oral doses of 2,000 mg/kg bw/day of DEHP in corn oil by gavage on days 15-17 of lactation. The rats were sacrificed 6 hours after the last dose. Increased activities of PCoA and CAT in dams and pups were observed also in this study. Mammary gland weights, both absolute and relative, was significantly reduced in treated rats. In treated rats, total milk solids, lipid, and protein were increased relative to control rats, whereas milk lactose was significantly decreased. Milk collected 6 hours after the third dose contained 216 µg/ml DEHP and 25 µg/ml MEHP. In contrast, plasma contained virtually no DEHP (< 0.5 µg/ml) but substantial amounts of MEHP (76 µg/ml) resulting in a high milk/plasma ratio for DEHP and a low milk/plasma ratio for MEHP. DEHP and MEHP were not detected in the plasma of the pups. After addition of (^{14}C) DEHP to milk *in vitro*, most of the radioactivity was associated with the fat globule layer.

In a study comparable to a guideline study, male Sprague-Dawley rats were given 10, 100, 1,000 or 2,000 mg/kg bw/day of DEHP (> 99% pure) in corn oil by gavage for 5 days beginning at an age of 6 (1-week-old), 14-16 (2-week-old), 21 (3-week-old), 42 (6-week-old) or 86 (12-week-old) days (Dostal et al., 1987b) (see also Section 4.1.2.9.1). The control group was given the vehicle. After two doses of 2,000 mg/kg bw/day virtually all pups in the three youngest age groups died whereas 6- and 12-week-old rats showed significantly decreased body weights with no fatalities. Five daily doses of 1,000 mg/kg bw/day caused significant decreases in body

weight gain in 1-, 2-, and 3-week-old rats. Absolute and relative liver weights were significantly increased at 100 mg/kg bw/day in all age-groups except in 1-week-old rats and in all age groups at higher dose levels. Absolute kidney weight was reduced in some cases whereas relative kidney weight was increased at doses of 1,000 mg/kg bw/day or more in 3-week-old rats or older rats. Morphological examinations revealed increased peroxisome proliferation in neonatal as well as adult rats. The activities of PCoA and CAT were increased in a dose-dependent manner in all age groups. The activities of these enzymes were similar in control rats of all ages. Plasma cholesterol concentrations were higher in suckling control rats (1- and 2-week-old) than in weanling (3-week-old) and adult controls. In DEHP-treated rats, plasma cholesterol concentrations were significantly reduced in weanling and adult rats given doses of 1,000 mg/kg bw/day or more. In suckling rats plasma cholesterol levels were increased at 1,000 mg/kg/bw/day. Plasma triglyceride levels in the control group were similar at all ages whereas significant decreases in plasma triglycerides were observed in weanling and adult rats; in suckling rats only small decreases (not significant) occurred.

Male Wistar rats (5 rats per group) were orally administered 250, 500, 1,000, or 2,000 mg/kg bw/day of DEHP (purity not specified) diluted in groundnut oil for 14 days (Khaliq and Srivastava, 1993). The control group was given the vehicle. Body weight gain was decreased in the two highest dose groups. Absolute liver weights were significantly increased in the two highest dose groups and relative liver weights in the three highest dose groups. The levels of liver polyamines were induced (spermidine 11-138% and spermine 3-78%) in a dose-dependent manner. The levels of spermidine increased more rapidly than those of spermine and the spermidine/spermine ratio was found to increase with increasing doses of DEHP. According to the authors, similar induction of polyamines occurred in tumour-bearing rats and they suggested that the increase in polyamine levels might be in correlation with the reported carcinogenic effect of DEHP.

The effects of DEHP on lipid metabolism were studied in male Sprague-Dawley rats (5-10 animals per parameter examined) given 1% DEHP (purity not stated) in the diet for 21 days (Bell et al., 1978). The relative liver weights were significantly increased. Hepatic total cholesterol and total lipid was not significantly different from control values. A significant reduction in serum cholesterol was seen in rats after 7 days of DEHP administration.

In a study of the effects of DEHP on the thyroid, male Wistar rats (18 rats per group) were fed diets containing 0 or 1% of DEHP (purity not specified) corresponding to approximately 1,000 mg/kg bw/day (Hinton et al., 1986). Six rats from the treatment group and six controls were sacrificed 3, 10, or 21 days after beginning of feeding. Serum thyroxin (T_4) and serum triiodothyronine (T_3) were measured. A significant decrease in serum levels of T_4 was observed whereas the levels of T_3 were unaffected (slight alterations observed were not significant). Electron microscopic examination of the thyroids of treated rats showed marked ultrastructural changes (increase in the number and size of lysosomes, enlargement of the Golgi apparatus, and damage of mitochondria) indicative of hyperactivity of the thyroid.

The neurobehavioural effects were tested in rats by a functional observational battery (FOB) and motor activity measurements before exposure, at specified times after a single dose exposure, and during and after a 14-day repeated dose exposure (Moser et al., 1995). Female Fischer 344 rats (number not given) were administered 150, 500, 1,500 or 5,000 mg/kg bw/day of DEHP (> 99% pure) (single dose study), or 50, 150, 500 or 1,500 mg/kg bw/day of DEHP (repeated exposure, 14 days) in corn oil by gavage. The FOB included following measures: autonomic, activity, excitability, neuromuscular, sensorimotor, and physiological measures. Motor activity was measured in a maze. The FOB was performed on each rat just prior to the first dose. Thereafter, the FOB followed by motor activity assessmentswas conducted at 4 and 24 hours

after exposure (single dose study), and on day 4 and 9 (before the daily dose) and 24 hours after the last dose. No lethality occurred. A single administration of the highest dose produced pronounced signs of general debilitation in two rats 24 hours after dosing. No changes in body weight were observed in either study. No functional domain was overall affected in either study.

Mice

In a study performed according to US-EPA Toxic Substances Control Act Test Guidelines 40 CRF 798 and to GLP principles, B6C3F1 mice (10 animals/sex/group) were fed 0, 1,000, 5,000, 10,000, or 25,000 ppm of DEHP (> 99% pure) in the diet for 4 weeks (Eastman Kodak, 1992b). Four male and three female mice from the highest dose-group died during the study. Compound-related clinical signs were observed only in the highest dose group. Body weights and body weight gains were significantly decreased in high-dose males and females and in males at the 5,000 ppm dose group. The weakly mean food consumption was significantly decreased at week 1 for males in the three highest dose groups and at week 2 for males in the highest dose group. Absolute and relative liver weights were significantly and dose-dependently increased in males and females fed 5,000 ppm DEHP or more. Hepatocellular hypertrophy (cytoplasmic enlargement, eosinophilia of hepatocytes around central veins) characterised as being moderate to severe was observed microscopically in high-dose mice. Focal coagulative necrosis occurred in males and females at the three highest dose groups. Absolute kidney weights were significantly decreased in males at the three highest dose groups. Relative kidney weights were significantly decreased in males at the 5,000 ppm group and in females at the 25,000 ppm group. Acute inflammation of the kidney (tubular necrosis, tubular dilatation with proteinaceous casts formation, tubular regeneration) occurred in mice at the three highest dose groups. Absolute and relative testes weights were significantly decreased in males of the two highest dose groups. Testicular atrophy was observed in males at the 25,000 ppm group. Other microscopic findings included thymic atrophy in mice of both sexes at the highest dose group and absence of corpora lutea in ovaries of females at the highest dose group.

Hamsters

Male DSN Syrian hamsters (5 animals per group) were given 25, 100, 250 or 1,000 mg/kg bw/day of DEHP (> 99% pure) by gavage in corn oil for 14 days (Lake et al., 1984b). Control hamsters (5 animals) received the vehicle. The relative liver weight was significantly increased in high-dose animals. No significant increase in the activities of PCoA and CAT was observed. Administration of similar doses of DEHP to rats resulted in a significant and dose-dependent (from 100 mg/kg bw/day) increase in liver weight as well as marked and dose-dependently increased activities of PCoA and CAT (see above).

Monkeys

In a study comparable to a guideline study and performed according to GLP principles, marmosets (5 animals of each sex) were given 2,000 mg/kg bw/day of DEHP (99.7% pure) by gavage in corn oil for 14 days (ICI, 1982b and Rhodes et al., 1986). The control group was given the vehicle. Body weight gains were unaffected. No effects were observed on liver and testis weights whereas the relative kidney weights were significantly reduced in females. Gross and microscopic examination of the liver, kidney, testes, and pituitary showed no changes. Electron microscopy revealed only a slight increase in peroxisomes. Plasma cholesterol and triglyceride levels were similar in treated and control animals. A significant increase in hepatic catalase activity was seen in males. In a comparative study in rats (ICI, 1982b, Rhodes et al., 1986 and see above), hepatomegaly, marked peroxisome proliferation, and an increase in the activity of

peroxisomal, mitochondrial and smooth endoplasmatic reticulum (microsomal) enzymes concerned with fatty acid metabolism were observed following a similar dosage regimen.

Male cynomolgus monkeys (one animal per group) were given 0, 100 or 500 mg/kg bw/day of DEHP (99.8% pure) by gavage in corn oil for 21 days (Short et al., 1987). There were no treatment related changes in relative liver weight, PCoA oxidation, CAT, or LAH-11 and LAH-12 hydroxylation. In addition, no treatment related effects were observed at light and electron microscopic examination of the livers. In contrast to these results, relative liver weight and PCoA oxidation, and LAH-11 and LAH-12 hydroxylation were significantly increased in male rats given doses from 6,000 ppm (667 mg/kg bw/day) or from 1,000 ppm (105 mg/kg bw/day), respectively, in the diet for 21 days. Peroxisome proliferation was moderate to very marked from 6,000 ppm.

The effects of DEHP as a peroxisome proliferator were evaluated in young adult male cynomolgus monkeys after 14 days of treatment, with emphasis on detecting hepatic and other effects seen in rats and mice after treatment with high doses of phtalater (Pugh et al, 2000). Monkeys weighing 2-2.9 kg were exposed to 500 mg DEHP/kg/day (dissolved in 0.5% methylcellulose) administrated in a constant volume of 10 ml/kg once a day for 14 consecutive days using an adult/pediatric nasogastric tube.

All animals were observed twice a day for mortality, morbidity, and toxicological or other clinical signs, including behavioural changes, appetite, and excreta.

Blood samples were collected during the second and fourth week of the pre-test period and prior to necropsy. Monkeys were sacrificed on the day following the last dose. A gross necropsy was performed including a thorough visual examination of all organs and body tissues. Organ weights were obtained for the liver, kidney, and testes/epididymis, adrenals, brain, heart, lung, spleen, and thyroid/parathyroid, and organ to body weight ratios were calculated. Sections of liver, kidney, and testes, were fixed and embedded for subsequent histopathological evaluation, assessment of replicative DNA synthesis by immunohistochemical detection, evaluation of GJIC and for assessment of peroxisomal activity.

There were no overt changes in the general health or behaviour of the monkeys following 14 days of dosing. Treatment with DEHP had no effect on body weights, food consumption, or relative weights of any organs assessed.

There were no changes in hematological parameters, serum chemistry or in the urine analysis.

No inflammation or necrosis was seen in any of the tissues examined.

The test substance did not produce any toxicologically important changes in the monkeys (Pugh et al., 2000)

Dermal

In the only available dermal study, DEHP (purity not specified) was percutaneously administered to DDY-mice (3 animals per dose group in a first experiment and 2 per group in a second) as daily doses of 0.2 ml of a 10, 30, 50, or 100% solution in olive oil for one month (Watari et al., 1978). Macroscopically, the liver was greatly enlarged, lacked elasticity, and had a dark brown colour. Inflammatory signs were observed in the peritoneum in the two highest dose groups. Structural alterations of the liver tissues were observed by light and electron microscopy and by cytochemistry in all dosed groups. In hepatic cells, the nuclei were atrophied and frequently contained fat droplets. The smooth endoplasmic reticulum was proliferated and vesiculated.

Mitochondria were decreased in size and some were degenerated. An unusual proliferation of the microbodies and their morphological alterations were found. The authors concluded that the results showed that DEHP is absorbed percutaneously and accumulates in the mouse liver resulting in damage to the cellular organelles. Due to several shortcomings, this study is, however, not adequate for use in the risk assessment.

Other Routes

A 3-day-old neonatal rat model was used to assess DEHP toxicity following intravenous administration (Greener et al., 1987). Neonates (12 rats per group, 2 to 4 days old) were injected 30.8, 91.7, or 164.8 mg/kg bw of DEHP (purity not specified) in 4% bovine serum albumin (BSA) solution for 18 consecutive days. Control neonates were injected a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1 to 3 hours later. After sacrifice, a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum, and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to 21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were detected in the tissues with the exception of local lesions at the injection site (subacute dermatitis), also noted in half of the BSA and saline control rats.

4.1.2.6.4 Subchronic toxicity studies (>28-days exposure <chronic exposure)

Oral

Rats

Fischer 344 rats (10 animals/sex/group, five- to six-week old) were given 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm of DEHP (> 99.5% pure) in the diet for 13 weeks to determine the high and low doses for a following chronic study (NTP, 1982). One male rat fed 6,300 ppm died. Depression of mean body weight gain of male and female rats fed 25,000 ppm was 29% and 55%, respectively, relative to controls. Testicular atrophy was observed in all males fed 25,000 ppm but was less pronounced in males fed 12,500 ppm (1,250 mg/kg/day). No other compound-related histopathological findings were observed.

In a study performed according to OECD guideline 408 and GLP principles, young male (105-130 g) and female (93-111 g) Sprague-Dawley rats (10 animals/sex/group) were administered 0, 5, 50, 500 or 5,000 ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw in males and 0.4, 4.2, 42.2 or 419.3 mg/kg bw/day in females) of DEHP (99.6% pure) in the diet for 13 weeks after a one-week acclimatisation period (Poon et al., 1997). No clinical signs of toxicity were observed. Body weight gain and food consumption were not affected. There were slight but significant decreases in red blood cell counts and serum haemoglobin, albumin, and potassium levels in male rats fed the 5,000 ppm diet. A reduction in the cholesterol concentration was observed in female rats of the same dose group. In the 5,000 ppm dose group, the liver was enlarged in 10 male and in 5 female rats and absolute and relative liver weights and relative kidney weights were significantly increased in rats of both sexes. Microscopic examination revealed minimal to mild hepatocellular hypertrophy in the liver from all rats of both sexes, minimal focal necrosis in one male and two females. Electron microscopy of liver samples revealed an increased number of peroxisomes in rats of both sexes. Significantly decreased

absolute and relative testicular weight and mild to moderate seminiferous tubule atrophy and mild to moderate Sertoli cell vacuolation in male rats (9/10) were also found at 5,000 ppm. In the thyroid**,** mild histological changes consisting of reduced follicle size and colloid density were detected in eight animals. In the 500 ppm dose group, the liver of one male rat was enlarged and minimal Sertoli cell vacuolation was observed in 7 male rats. The NOAEL for the testicular effects (based on Sertoli cell vacuolation in male rats) was considered to be 50 ppm DEHP in the diet (3.7 mg/kg bw/day). A NOAEL for the effects on the kidney, can be considered to be 500 ppm (37.6 mg/kg bw in males).

In a study performed according to US-EPA Toxic Substances Control Act Test guidelines 40 CFR 798 and according to GLP principles, Fischer 344 rats (10 animals/sex/group) were fed 0, 1,000, 4,000, 12,500 or 25,000 ppm of DEHP (99.7% pure; corresponding to 0, 63, 261, 850, or 1,724 mg/kg bw/day in males and 0, 73, 302, 918 or 1,858 mg/kg bw/day in females) in the diet for 13 weeks (Eastman Kodak, 1992a). All animals survived throughout the study. The body weight gains were significantly decreased in high-dose animals of both sexes and in females given 12,500 ppm. Absolute and relative liver weights were significantly and dose-dependently increased in all treated animals except for the 1,000 ppm females. Hepatocellular enlargement was observed microscopically in animals of both sexes from the two highest dose groups and in males from the 4,000 ppm dose group. Relative kidney weights were significantly increased in the two highest dose groups and in the 4,000 ppm male group. In males of the highest dose group, testis and epididymides weights were significantly decreased; atrophy of testes and epididymal aspermia were observed histologically. The identified LOAEL of 1,000 ppm (63 mg/kg/day in males and 73 mg/kg/day in females) corresponds to the lowest dose level used in this study.

In two separate studies with exposure duration of 9 weeks or 4 weeks, male Wistar rats were dosed with DEHP by gavage and exposed to drinking water with or without acetone (0.5% wt/v in the 9-week study, 1% wt/v in the 4-week study)(Dalgaard M. et al, 2000). 2-ethylhexanol is a metabolite of DEHP, which role is not fully elucidated. Both the phtalic acid and the aliphatic (ethylhexyl) part of DEHP may be toxic to the testis. The possible contribution of the aliphatic part to the testis toxicity can be examined by administering acetone concomitantly with DEHP.In the 9-week study the doses of DEHP were 0, 125, 500 or 1,000 mg/kg bw with soya oil as a vehicle in a dose volume of 2 ml/kg bw. In the 4-week study the doses of DEHP were increased to 1,000, 5,000 and 10,000 mg/kg bw. In each study 80 male rats were used; 10 animals per group, weighing approximately 160g. In the 4-week study additionally 80 female rats, approximately 10 weeks of age, were used to investigate male fertility. The rats were housed two per cage in wire cages with a 12-hour reversed day:night cycle. They were given a standard diet and they were observed twice a day for clinical signs of toxicity. During both studies body weight was measured once a week. Food and water consumption per cage was registered on a weekly basis. The rats were tested in a Functional Observational Battery (FOB). On the day before sacrifice, rats were anaesthetised and blood was collected for clinical biochemical analyses. During the last week of the 4-week study all male rats from each dose group were mated with undosed females. On day 15 after mating or at the end of the allocated mating period, the female rats were sacrificed, and the uteri and ovaries removed. The uterus was opened and the number of implantations and dead or retarded foetuses was counted. All males from each group underwent a thorough autopsy. Liver, kidneys, adrenals, heart, spleen, testes, epididymis, seminal vesicles, and brain were excised and weighed. In the 9-week study the histopathology was only performed in the control group and in the group receiving the highest dose level of DEHP. Histopathological findings of the testes were graded into normal, slight/moderate atrophy, or severe atrophy. In the 4-week study immunohistochemical investigations were performed on testis vimentin.

In the 9-week study no animal died and no other clinical abnormalities were observed. No effect on body weight, food and water consumption was observed. No histopathological changes were observed in any of he investigated organs of he control and animals exposed to 1,000 mg/kg bw DEHP. The only effect of DEHP was the statistically significant increase in relative liver weight in the dose group exposed to 500 and 1,000 mg DEHP/kg bw with or without acetone in the drinking water.

In the 4-week study a statistically significant reduction in body weight and food and water consumption was observed in the middle and high dose groups. This reduction was getting more pronounced during the study and was dose-dependent for DEHP. Six animals died due to emaciation, two animals in the group dosed with 10,000 mg/kg bw DEHP and four animals dosed with the same amount of DEHP combined with 1% acetone in the drinking water. No differences were found between groups in the FOB test. Hindlimp grip strength was statistically significantly reduced in the groups receiving acetone compared to the groups not receiving acetone. Forelimb grip strength was statistically significantly reduced with increasing doses of DEHP. There was a statistically significant reduction in the level of cholesterol in animals dosed with 125mg, 500 or 1,000 mg DEHP/kg bw and in animals exposed to 250 mg DEHP/kg bw in combination with 0.5% acetone.

The number of males without recognised mating increased in a dose-related manner although the effect was only statistically significant in the group receiving 10,000 mg DEHP/kg bw plus 1% acetone (4/6). The number of pregnant females decreased with increasing DEHP dose levels (9/10→1/8). The most pronounced effect of DEHP was a statistically significant increase in relative liver weight, which was observed in treated animals. In the middle and high dose groups body weight was statistically significantly reduced, while the relative weights of kidney and brain were statistically significantly increased. Testes weight was statistically significantly reduced in the groups exposed to 5,000 and 10,000 mg DEHP/kg bw with or without acetone. The weight of epididymals and seminal vesicles were statistically significantly reduced at the highest dose level. DEHP had no effect on the weight of spleen. The testis of two rats dosed with 5,000 mg/kg bw showed severe atrophied tubules with massive loss of spermatids and spermatocytes sloughed into the lumen. In most tubules the majority of the germinal epithelium was lined by spermatogonia and Sertoli cells or by Sertoli cells alone. 5 animals that received 5,000 mg DEHP/kg bw + acetone had some microscopic features of severe atrophy of the testes and occasionally thickening of the basal lamina of the tubule. Slight to moderate atrophy was seen in two animals: one dosed with 10,000 mg DEHP/kg bw and one dosed with 5,000 mg DEHP/kg bw + 1% acetone. All other surviving animals dosed with 10,000 mg DEHP/kg bw with or without acetone in the drinking water showed severe seminiferous tubular atrophy. A slight diffuse Leydig's cell hyperplasia was observed in testes with severe atrophy.

Mice

B6C3F1 mice (10 animals/sex/group) were given 0, 800, 1,600, 3,100, 6,300 or 12,500 ppm of DEHP (> 99.5% pure) in the diet for 13 weeks (NTP, 1982). Seven out of 10 mice in the highest dose group died; these deaths were, however, accidental. Two female mice in the 3,100 ppm group and one female in each of the control, 6,300 and 12,500 ppm groups died. A mean body weight gain depression of 10% or more was observed in males fed 3,100 ppm and more and in all female dose groups except for those fed 1,600 ppm. No other compound-related effects were observed.

Rabbits

Rabbits were given DEHP (purity not specified) by gavage in olive oil (BASF, 1961). Three female rabbits and three male rabbits died following administration of 4-5 doses of 2 ml/kg bw/day (1,960 mg/kg bw/day) and 1 ml/kg bw/day (980 mg/kg bw/day), respectively. Two male rabbits died following administration of 24 or 31 doses of 0.5 ml/kg bw/day (490 mg/kg bw/day) whereas one female rabbit in this dose group survived administration of 50 doses. One female rabbit died following administration of 9 doses of 0.2 mg/kg bw/day (196 mg/kg bw/day) whereas the two other females in this dose group survived 50 and 51 doses, respectively. Pathological examination revealed pneumonia, liver and kidney lesions. In the control groups (given 1 or 2 ml/kg bw/day of olive oil), the two high-dose rabbits died after 8 and 52 application, respectively; the two low-dose rabbits died after 32 and 50 applications, respectively. Pathological examination revealed pneumonia and necrosis of the liver in 3 out of 4 animals. No further details are available. The quality of this study is not considered adequate for use in the risk assessment as the results are very briefly reported, few animals were examined, and similar effects were observed both in the controls and in the treated groups.

Cats

Three cats were given 60 daily doses of 1 or 2 ml DEHP/kg bw (980 or 1,960 mg/kg bw/day; purity not specified) in olive oil by gavage (BASF, 1961). None of the cats died. Reduced food intake, weight loss, diarrhoea, and vomiting were observed. Pathological and histological examinations of the low-dose animals revealed bronchitis, bronchopneumonia, and in one cat hepatitis and kidney lesions. Similar pathological findings were observed in the vehicle control animals. No further details are available. The quality of this study is not considered adequate for use in the risk assessment as the results are very briefly reported, few animals were examined, and similar effects were observed both in the control group and in the treated groups.

Monkeys

In a 13-week oral study performed according to GLP principles, marmosets (4/sex/group) were daily administered 0 (corn oil), 100, 500 or 2,500 mg/kg DEHP (purity not specified) in corn oil (Kurata et al., 1995). Males were dosed from 13 to 14 months of age and females from 12 to 15 months of age.

The body weight gain was significantly suppressed in males administered 2,500 mg/kg. There was a significant decrease of the absolute weight of the spleen in dosed males and also a similar trend for the relative weight; this was thought to be of little toxicological significance as no histopathological changes were found. Other organ weights, including liver, testes, and pancreas, were not different from the control weights. In the DEHP dosed groups there was a significant rise in the total and free cholesterol and phospholipid levels in administration week 4. In week 13, only the total cholesterol value in the 500 mg/kg males was different from the control value. It could not be concluded that the effect was caused by the administration of DEHP. A clear rise in blood testosterone and oestradiol concentrations in all groups, including controls, were concluded to be hormonal changes accompanying sexual maturity occurring at the age of about 12 months. In the 500 and 2,500 mg/kg group males, a significant increase in the average hepatic peroxisome area was observed, but there was no difference from the control group in terms of the number of peroxisomes per cell or the area density with respect to the cell area of the peroxisomes. No difference from the control group was seen concerning hepatic peroxisome enzyme activities in the DEHP dosed groups. A rising trend (not statistically significant) in the hepatic microsome protein content and an accompanying rising trend in the cytochrome P-450

content per unit liver weight were observed in all dosed males and in mid- and high-dose females.

The effects on various organs of long-term oral administration of DEHP have also been studied in marmosets (Kurata et al., 1996; 1998). Marmosets (12-15 months old; 4 animals per sex and group) were given daily doses of 0, 100, 500 or 2,500 mg/kg bw of DEHP (purity not specified) by gavage in corn oil for 13 weeks. The control group was given the vehicle. A significant suppression of body weight gain was observed in high-dose males. Dose-related decreases in spleen weight were observed in all dosed males. Light and electron microscopic examination revealed no substance-related abnormality in the liver in any dosage group. A slight but significant increase in the mean hepatic peroxisome volume was observed in mid- and high-dose males, but the number of peroxisomes and their volume density was not different from those in the control group. No substance-related change of peroxisome-related enzyme activity (catalase, CAT, and PCoA) was observed in any dosage group. No effects on the testes were seen.

4.1.2.7 Chronic Toxicity studies (more than 10% of the test animals lifespan)

4.1.2.7.1 *In vivo* studies

Inhalation

Mice

Young male ICR mice (20 animals) were exposed to air saturated with vapours of DEHP (purity not specified) for 2 hours per day, 3 days per week, for 4-16 weeks (Lawrence et al., 1975). Control mice were similarly exposed to air. At the end of 4, 8, 12, and 16 weeks, five mice from each group were sacrificed. None of the mice died during the study. The lungs and several other tissues were examined histopathologically. The histological examination of the tissues, particularly the lungs, failed to reveal consistent abnormalities which could be attributed to inhalation of vapour of DEHP. No further data are available. This study is considered to be of a limited quality with respect to risk assessment of adverse effects following inhalation of DEHP as the documentation is insufficient for an assessment.

Hamster

The only long-term inhalation study available (Schmezer et al., 1988, see **Table 4.39**) is on hamsters, and is considered inadequate for risk assessment as:

- only one dose of DEHP was used in the study.
- this dose was very low (continuos inhalation of 15 $\mu g/m^3$ for 23 months). According to the authors: “The aim of the study was to use dose range of environmentally relevant exposure similar to the human situation in indoor circumstances, and much higher concentrations may occur at some work places for short time periods”
- MTD was not reached as no signs of any toxicological effects were reported.

Oral

Rats

Male Fischer 344 rats (18 animals per group) were fed a diet containing 0 or 1.2% DEHP (purity not specified) (Eagon et al., 1994). After 4, 8, and 16 weeks of treatment 6 animals from the treatment group and the control group were sacrificed. Body weight was significantly decreased after 4 weeks, but not after 8 and 16 weeks. The liver weight was significantly increased at all exposure times. The concentrations of total lipids, cholesterol, cholesterol esters and triglycerides in the serum were significantly decreased after 4 and 8 weeks, but not after 16 weeks. Oestrogen receptor activity was significantly reduced in cytosolic and nuclear fractions from livers of rats treated for 8 and 16 weeks. Serum oestradiol was significantly elevated at all exposure times. Hepatic microsomal oestrogen 2-hydroxylase was significantly reduced after 4 and 8 weeks, and slightly but not significantly after 16 weeks. Male oestrogen binding protein and oestrogen receptor mRNA were significantly decreased after 8 weeks. According to the authors, the observed changes in hepatic oestrogen metabolism together with the induced hyperplasia could play a crucial role in the hepatocellular carcinogenesis induced by DEHP in rats.

In a study comparable to a guideline study, Sprague-Dawley rats (15 animals/sex/group) were given diets containing 0, 0.2, 1.0 or 2.0% DEHP (purity specified as conforming with British Standards Institution) (0, 143, 737 or 1,440 mg/kg/day in males and 0, 154, 797, or 1,414 mg/kg bw/day in females) for 17 weeks (Gray et al., 1977). Rats in the highest dose-group showed clinical signs of toxicity (loss of fur) from week 1 through week 17 of treatment. The body weights of rats in the two highest dose groups were reduced compared to controls, significantly from day 2 in both sexes given 2% DEHP and from day 6 or day 83 in males or females, respectively, given 1% DEHP. The body weights of low-dose rats were lower (not significant) than in controls. Food intake was significantly reduced only in rats given 2% DEHP. Both absolute and relative liver weights were significantly increased in all dose groups when compared to controls. The absolute weights of most other organs (including testes) in rats of the two highest dose groups were lower than those of controls while the relative weights were increased. Histological examination revealed severe seminiferous tubular atrophy and cessation of spermatogenesis which could be related to the dietary level of DEHP. These changes were evident as early as week 2. No histological changes attributable to DEHP treatment were observed in the livers. No NOAEL can be identified from this study; the LOAEL corresponds to the lowest dose level, 0.2% in the diet (143 mg/kg/day in males and 145 mg/kg/day in females).

Chu et al. (1981) studied the toxicity of MEHP in male Sprague-Dawley rats (6 animals per dose group) administered single oral doses of 50, 100, or 200 mg/kg bw of MEHP (> 99% pure) or 2,000 mg/kg bw of DEHP (> 99% pure) by gavage in 2% gum acacia. The control group received the vehicle. Body weight and food consumption were not affected. Relative liver weight was significantly increased in mid- and high-dose animals given MEHP and in animals given DEHP.

In a subsequent 28-day study, male weanling Sprague-Dawley rats (10 animals per group) were fed diets containing 0, 25, 100, 400, 1,600 or 6,400 ppm of MEHP **(**Chu et al., 1981). Major organs were excised and weighed (including testes). At 6 400 ppm a significant growth retardation was noted. Liver and heart weight were significantly increased from 1,600 ppm. Histological examination revealed, however, no treatment-related abnormalities. In the following 3- and 6-month feeding studies, weanling Sprague-Dawley rats (20 animals/sex/group) were fed diets containing 0, 1, 5, 25, 125 or 625 ppm of MEHP. After 3 months, 10 animals of each sex from each group were necropsied. The remaining animals were maintained on the same diet and

sacrificed after six months. Relative organ weights were not altered in the 3-month period, but the liver weights of high-dose females were significantly increased in the 6-month period. Treatment-related lesions were found in the liver (midzonal and periportal eosinophilic cytoplasmic inclusions, and vacuolations with isolated binucleated and necrotic hepatocytes), heart (mild enlargement of myocardial nuclei and segmental deregistration of myocardial striations), and adrenals (vacuolation of the zona fasciculata).

In a study comparable to a guideline study and performed according to GLP principles, groups of male and female Alderley Park rats (20 animals/sex per treatment group, 30 animals/sex in the control group) were fed diets containing sufficient DEHP (> 99.7% pure) to ensure intakes of 0, 50, 200 or 1,000 mg/kg bw/day (CEFIC, 1982; Mitchell et al., 1985a). Four rats from each treatment group and six controls and were sacrificed 3, 7, 14, 28 days, and 9 months after beginning of feeding. No clinical signs of toxicity were observed during the study. Food consumption of the treated rats was either similar to or greater than that of controls. Body weight was significantly reduced only in rats given 1,000 mg/kg bw/day for 9 months. Liver weights were increased in male rats fed 50 and 200 mg/kg bw/day for 14 days or more and in all high-dose rats at all time intervals. Examination of liver sections by light microscopy showed periportal accumulation of fat and mild centrilobular loss of glycogen, both effects were dose-dependent. Electron microscopy revealed that the number of peroxisomes was significantly increased at 50 mg/kg bw/day after 14 days in males and after 9 months in females. Higher doses caused increased number of peroxisomes after 3 days in males and after 14 days in females, the increase was dose-dependent in both sexes. Dose-dependent alterations to the endoplasmatic reticulum (ER) was also observed. Smooth endoplasmatic reticulum proliferation was significantly increased at 50 mg/kg bw/day after 7 days in males and after 14 days in females. Changes in rough endoplasmatic reticulum were observed at 200 mg/kg bw/day after 3 days in males and after 28 days in females. The density of the mitochondrial matrix was increased in male rats although there was no dose dependence. Biochemical studies revealed a marked induction of the peroxisomal enzymes PCoA and α-GD; the induction was dose and time-dependent. The effects in female rats increased more slowly than in male rats but were equivalent by 28 days. The ER-associated enzymes cytochrome P-450 and LAH were dose-, but not time-dependently increased with maximal activity observed at 3 days of treatment. No effect on testis weight was observed. Alterations of the kidneys (lysosomes in the cells of the proximal tubule were enlarged) were observed at 200 and 1,000 mg/kg bw/day. In thyroids of rats fed 1,000 mg/kg bw/day for 9 months showed also alterations (basophilic deposits in the colloid and enlargement of the lysosomes).

Male Fischer 344 rats (5-10 animals per group) were fed 1.2% DEHP (purity not specified) in the diet for 1, 2, 4, 8, 18, 39, 77, 151 or 365 days (Conway et al., 1989). Livers were examined biochemically. Catalase activity was increased (25%) after 8 days and remained at this level up to 365 days. Glutathione peroxidase activity showed a 50% and 80% decrease after 8 or 365 days of treatment, respectively. Lipofuscin, which was contained within lysosomes, was increased 3-fold after 39 days and remained at this level up to 365 days of treatment. The activities of the lysosomal enzymes α-fucosidase, ß-galactosidase and N-acetylglucosaminidase were increased 50-100% for 39-365 days of treatment.

In a study on male rats (Crocker et al., 1988), the animals were treated by gavage, three times weekly, with DEHP, a leachate resulting from toluene extraction of a phthalates-containing artificial kidney, or sesame oil. 65 animals were divided into 3 groups: (a) 150 mg DEHP/ 70 kg bw, approximately 0.9 mg/kg bw/day, (b) 150 mg leachate/70 kg bw, approximately 0.9 mg/kg bw/day or (c) sesame oil controls. Four rats from each group were sacrificed at 3, 6, 9 and 12 months and their kidneys were removed for histopathologic examination and phthalate

analysis. Also, at each of these intervals, a 24-hour urine and blood samples were collected to determine creatinine clearance. A statistically significant increase ($p = 0.04$) in the incidence of focal cystic changes was observed in kidneys of rats received DEHP or leachate and killed at 12-month interval. Creatinine clearance was significantly decreased ($p = <0.01$) only in rats receaved pure DEHP and killed at the 12-months interval.

In a more recent long-term study, comparable to a guideline study and conducted according to the principles of GLP (Moore, 1996), F-344 rats were administered DEHP at dietary concentrations of 0, 100, 500, 2,500 or 12,500 ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day, respectively, for males, and 0, 7.3, 36.1, 181.7 or 938.5 mg/kg/day, respectively, for females),70 males and females/group, for at least 104 weeks (see Section 0). An additional group was administered 12,500 ppm DEHP for 78 weeks, followed by a recovery period of 26 weeks.

There were no treatment-related effects at 100 and 500 ppm. At 2,500 ppm the mean serum albumin concentration and mean liver weights were significantly increased. At Week 79 and at study termination also absolute and relative kidney weights were increased in both sexes at 2,500 ppm. At the highest dose level, there was a decreased survival, increased incidence of clinical abnormalities, and decreased body weight gain in both sexes. A diffuse hepatomegali and histopathological hepatic changes were demonstrated as were effects on the kidneys, including increased absolute and relative kidney weights (both sexes), increased incidence and severity of mineralisation of the renal papilla in males, increased incidence and/or sseverity of tubule cell pigment in both sexes, and increased severity of chronic progressive nephropathy in the males. In the males, also absolute and relative testis weights were significantly decreased at 12,500 ppm, with associated increased incidence of bilateral aspermatogenesis, and decreased incidence of interstitial cell neoplasms. In the pituitary, an increased number of castration cells were observed in 30/60 males compared to 1/60 of the control males. There was no indication in rats killed at study termination that DEHP-related changes in the kidney, testis, and pituitary were reversible upon cessation of DEHP-exposure. The NOAEL for systemic non-neoplastic effects, including the effects on the kidney is considered to be 500 ppm DEHP in the diet (corresponding to 28.9 mg/kg bw/day in the males and 36.1 mg/kg/day in the females) based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL: 2,500 ppm corresponding to 146.6 mg/kg bw/day in the males and 181,7 mg/kg bw/day in the females).

In a two years-study, Fischer 344 rats (50 animals/sex/group) were given 0, 6,000 or 12,000 ppm of DEHP (> 99.5% pure) in the diet. (NTP, 1982) Mean daily ingestions of DEHP were calculated to 322 and 674 mg/kg bw/day for low- and high-dose male rats, respectively, and to 394 and 774 mg/kg bw/day for low- and high-dose female rats, respectively. The survival rate was unaffected. At the end of the study, mean body weights of dosed male rats and high-dose female rats were marginally to moderately lower than those of the corresponding controls. Food consumption was slightly reduced in rats of either sex. In high-dose males, the incidence of hypertrophy of the anterior pituitary was significantly increased (45%); and in the testis, degeneration of the seminiferous tubules occurred in 90% of the animals (for details see Section 0 Carcinogenicity and the effects on the liver are described in detail in Section 0.

Male Sprague-Dawley rats (a total of 520 animals) were fed 0, 0.02, 0.2 or 2% DEHP (0, 7, 70 or 700 mg/kg bw/day; > 99% pure) in the diet for 102 weeks (Ganning et al., 1987, 1990). The body weights were significantly reduced in the highest dose group reaching 20% lower values after 25 weeks of treatment. Significantly reduced body weights were also observed in the mid-dose group but to a lesser extent (around 10%). Electron microscopy revealed characteristic changes in hepatocytes. After one week of treatment with 2% DEHP, peroxisome proliferation (varying size) was observed and was persistent throughout the study. The number of

mitochondria was increased whereas no changes were observed in rough and smooth endoplasmatic reticulum. After feeding with 0.2% DEHP for 16 months, an increased number of peroxisomes and mitochondria and well-developed endoplasmic reticulum were observed. The number of peroxisomes and mitochondria was not significantly increased after feeding of 0.02% DEHP when compared with the control group. The activity of PCoA was significantly increased in all dose-groups with a doubling of the activity in the lowest dose group after 2 years of treatment. This long-term treatment with DEHP had complex effects on catalase activity with decreasing activity during the initial phase, at 10 weeks the activity exceeded that of the control, but returned to normal at the end of the study. The activity of urate oxidase decreased throughout the study. No hyperplastic nodules or primary liver carcinoma or other tumours were observed. In all dose-groups, DEHP exerted a pronounced effect on the function of the testes after prolonged treatment, consisting of inhibition of spermatogenesis and general tubular atrophy. No NOAEL can be derived from this study; the LOAEL is 0.02% in the diet (7 mg/kg bw/day), the lowest dose administered. In a following study investigating the reversibility of the observed effects, rats were fed a diet containing 2% DEHP for 1 year and then the basal diet for 3 weeks. PCoA and catalase activities decreased after cessation of treatment and reached control levels after 2 weeks.

Five male Sprague-Dawley rats were fed 2% DEHP (> 98% pure) in the diet for 2 years (Lake et al., 1987). The control group consisted of 8 male rats. The relative liver weights were significantly increased compared to control values. Morphological changes in form of liver nodules were observed. Ultrastructural examination of both non-nodular and nodular areas from treated rats showed a marked increase in the number of mitochondria and a smaller increase in the number of peroxisomes. Hepatic peroxisomal and microsomal fatty acid oxidising enzyme activities were induced whereas little change in catalase activity was observed. The activities of cytosolic GSH peroxidase and GSH S-transferases were markedly reduced. Increased lipid peroxidation (up to 6-fold) was observed in the treated rats. According to the authors, these results demonstrate that prolonged peroxisome proliferation can result in lipid peroxidation and that certain enzymes which metabolise hydrogen peroxide and organic hydroperoxides are either little affected or markedly inhibited.

Mice

In a study comparable to a guideline study and performed according the principles of GLP (Moore, 1997) B6C3F1 mice (70-85 of each sex/dose group) were administered DEHP daily in the diet at concentrations of 0, 100, 500, 1,500 and 6,000 ppm (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg/day, respectively, for males, and 0, 23.8, 116.8, 354.2 or 1,458.2 mg/kg/day, respectively, for females), for 104 weeks (see also Section 4.1.2.9.1). One additional group (55 males and females/group) were administered 6,000 ppm DEHP for 78 weeks, followed by a 26-week recovery period. At 1,500 ppm, there was a significant decrease in kidney weight in males and an increased incidence and/or severity of chronic progressive nephropathy in both sexes. The testicular weight was also significantly decreased, with an increased incidence and severity of bilateral hypospermia and an associated increased incidence of immature/abnormal sperm forms and hypospermia in the epididymis. At the highest dose level, there was a statistically significant decrease in survival in males, treatment-related clinical signs and a significantly reduced body weight gain for both males and females. In both males and females, the kidney weight indices were significantly decreased at study termination. In the recovery group, some treatment-related findings were reversible or did not progress after cessation of DEHP exposure such as effects in the liver, including induction of neoplasms (described in detail in Section 0). In contrast to the liver, the effects of DEHP on the kidney and testis were not reversible following cessation of exposure. The NOAEL in this study is considered to be

500 ppm (due to effects on the kidney at the next higher dose level) corresponding to 98.5 mg/kg in males and 116.8 mg/kg in females.

In a study performed according to GLP principles, B6C3F1 mice (50 animals/sex/group) were given 0, 3,000, or 6,000 ppm of DEHP (> 99.5% pure) in the diet for 103 weeks (NTP, 1982). Mean daily ingestion of DEHP was calculated to 672 and 1,325 mg/kg bw for low- and high-dose males, respectively, and to 799 and 1,821 mg/kg bw for low- and high-dose females, respectively. The survival rate was unaffected in male mice but several female mice in the lower dose group died after 75-90 weeks of treatment. These deaths were not attributed to DEHP administration. Mean body weights of female mice were marginally to moderately lower than those of the corresponding controls at the end of the study. A significantly higher incidence of chronic inflammation of the kidney was observed in high-dose males. Bilateral seminiferous tubular degeneration and testicular atrophy were observed in 14% of the male mice in the high-dose group. This lesion was also found in one control male mouse and in two low-dose males. Non-neoplastic lesions were not observed in female mice. Effects on the liver are described in detail in Section 4.1.2.9.1.

Guinea pigs

Guinea pigs (22-24 animals/sex/group) were given 0, 0.04% (19 mg/kg bw/day), or 0.13% (64 mg/kg bw/day) of DEHP (purity not specified) in the diet for one year (Union Carbide, 1950). The mortality was 11/46, 14/46 and 7/47 in the control group, and in the 0.04% and 0.13% dose groups, respectively. Around 60% of the deaths were attributed to infections of the lung. The body weights were significantly increased in the low dose animals. Food consumption was significantly increased in animals of both dose groups. Relative liver weights were significantly, but not dose-dependently, increased in dosed females but not in males. Kidney weights were unaffected. There were no gross or microscopic alterations in the liver, kidney, or lung.

Dogs

Dogs (2 animals of each sex) received 0.06 ml DEHP/kg bw/day (purity not specified) (59.1 mg/kg bw/day) in gelatine capsules 5 times weekly for one year (Union Carbide 1951). The control group (2 animals of each sex) were given gelatine capsules without DEHP. No significant differences were observed between treated dogs and control dogs in the following parameters: mortality, body weight gain, blood counts, and gross and microscopic examination of organ and tissues (liver, kidney, lung, heart, stomach, upper and lower intestine, caecum, spleen, adrenal, ovary or testes, bladder, and thyroid).

Other Routes

Monkeys

The effects of DEHP on hepatic function and histology were evaluated in the rhesus monkey undergoing longterm platelet and plasma transfusion (Jacobson et al., 1977 and Kevy and Jacobson, 1982). Three monkeys were transfused weekly for one year with 15 ml of PVC platelet-rich plasma (PRP) stored for 48 hours at 22°C (total dose of DEHP: 24.4, 30.0, or 26.2 mg/kg bw/monkey, respectively) and two monkeys received PRP stored at 4°C (total dose of DEHP: 8.7 or 6.6 mg/kg bw/monkey, respectively). Another two monkeys were transfused bi-weekly for 6 months with 15 ml of platelet-poor plasma (PPP) stored for 5 days at 22°C (total dose of DEHP: 30.0 or 33.0 mg/kg bw/monkey, respectively). Three monkeys were used in an

untransfused control group and two monkeys were transfused PRP stored for 48 hours at 22°C in polyethylene blood bags. Four out of the seven PVC-transfused monkeys had abnormal ^{99m}Tc Sulphur Colloid Liver-Spleen Scan ratios (severe or moderate impairment of hepatic perfusion and/or infiltration are detected by irregular distribution of the tracer within the liver). Four out of seven monkeys demonstrated abnormal sulfobromo-phthalein clearance indicative of subclinical liver disease. Sex out of the seven had abnormal liver histology (aggregates of inflammatory cells, hepatocyte degeneration, and multi- and bi-nucleated giant cells) upon completion of transfusion period. None of the six control animals had abnormal liver histology. These findings persisted in three of the five surviving monkeys throughout the follow-up period of 26 months. The nontransfused monkeys and those transfused with platelets stored in polyethylene bags were normal. None of the monkeys had reached sexual maturity at study termination. All seven PVC-transfused rhesus monkeys showed abnormal liver histology

4.1.2.7.2 *In vitro* studies

The effects of the metabolites of DEHP have been studied in primary hepatocyte cultures of rat, rabbit, guinea pig, and monkey (Dirven et al., 1993c). The cell cultures were derived from male Wistar rats, male Dunkin Hartley guinea pigs, male New Zealand rabbits, and male cynomolgus monkeys and cultured in the presence of various concentrations of MEHP (> 99% pure), mono(5-carboxy-2-ethylpentyl) phthalate (metabolite V) (purity: 96%), or mono(2-ethyl-5-oxohexyl) phthalate (metabolite VI) (purity: 99%). In rat hepatocyte cultures, MEHP and metabolite VI were equally potent in inducing peroxisome proliferation (50% increase in peroxisomal palmitoyl-CoA oxidase activity and microsomal lauric acid ω-hydroxylation activity with 5-15 μM MEHP), while metabolite V was much less potent. In hepatocyte cultures derived from guinea pigs, rabbits, and monkeys, a 50% increase in peroxisomal palmitoyl-CoA oxidase activity was found after treatment with 408-485 μM MEHP. No induction of microsomal lauric acid ω-hydroxylation activity was found in these three species.

The effects of the DEHP metabolite mono(2-ethyl-5-oxohexyl) phthalate (metabolite VI) (purity not specified) have also been studied in primary hepatocyte cultures of male Alderley Park rats, Dunkin Hartley guinea pigs, and humans (Elcombe et al., 1997). Metabolite VI increased peroxisomal fatty acid β-oxidation in cultured rat hepatocytes, similar exposures of guinea pig or human hepatocytes did not provoke an induction.

A primary rat hepatocyte culture system was utilised to determine the proximate peroxisome proliferator(s) derived from DEHP (Mitchell et al., 1985b). DEHP (purity not specified) was administered to rats by gavage and the urinary metabolites were identified and isolated. The major metabolites of the ω-oxidation pathway [mono(5-carboxy-2-ethyl-pentyl) phthalate (metabolite V) and mono(3-carboxy-2-ethylpropyl) phthalate (metabolite I)] and of the (ω-1)-oxidation pathway [mono(2-ethyl-5-hydroxyhexyl) phthalate (metabolite IX) and mono(2-ethyl-5-oxo-hexyl) phthalate (metabolite VI)] together with MEHP and 2-EH were added to primary rat hepatocyte cultures and the effect on peroxisomal enzyme activity was determined. The (ω-1)-oxidation products (metabolite V and IX) produced a large (7- to 11-fold) induction on PCoA oxidation whereas the ω-oxidation products (metabolite I and V) produced little or no effect. Similar effects were observed for the induction of cytochrome P-450-mediated LAH.

4.1.2.7.3 Evaluation and conclusions of repeated dose toxicity

Limited human data are available.There is, however, a study suggesting that toxic damage of the lungs in preterm infants artificially ventilated with PVC respiratory tubes may be causally related to inhalation of DEHP. The estimated inhalative exposure ranged between 1 µg/h – 4,200 µg/h DEHP. Other studies, one morbidity and one mortality study, and three epidemiological studies conducted on workers exposed to DEHP and other phthalate esters are considered inadequate with respect to the risk assessment.

In experimental animals three inhalation studies are available. A 4-weeks study in rats (BASF, 1990 and Klimisch et al., 1992), a 4-16 weeks study in mice (Lawrence et al., 1975) and a 23-month study in hamster (Schmezer et al., 1988). However, these studies are considered inadequate for risk characterization (for details see Section 4.1.2.6.23 and 4.1.2.7.1).

The only study available following dermal exposure to DEHP is inadequate for risk assessment.

Numerous studies have investigated the toxicity of DEHP following repeated oral administration to experimental animals, preferably rats. Many of these studies are comparable to guideline studies and conducted in conformity with GLP. Critical organs for DEHP induced toxicity in laboratory animals are the, testis, kidney, and liver.

Testicular effects are discussed in more detail in Section 4.1.2.1010.

The effects on the kidneys include: reduced creatinine clearance, increased absolute and relative kidney weights, increased incidence and severity of mineralization of the renal papilla, increased incidence and/or severity of tubule cell pigment, and increased incidence and/or severity of chronic progressive nephropathy. The majority of these changes were observed in both sexes, in different species following different exposure time. In long-term studies in rats and mice (Moore, 1996 and 1997), there was no indication that DEHP-related changes in the kidney were reversible upon cessation of DEHP-exposure.

In a one-year study on male rats (Crocker et al., 1988), an increase in the incidence of focal cystic changes in kidneys ($p = 0.04$), and a decrease in Creatinine clearance ($p = 0.01$) was observed in rats receiving very low doses of DEHP (approximately 0.9 mg/kg bw/day). In clinical chemistry, reduced creatinine clearance is used as a validate indicator for disturbance in kidney functions. Therefore, 0.9 mg/kg bw/day DEHP may be regarded as the lowest LOAEL for kidney toxicity in rats, especially when related to the more severe kidney effects observed at higher levels of DEHP in a number of studies conducted with different animal species. However, in Crocker's study only few animals were used (4 rats/group), and the reported data is not always clear. As the effect on creatinine clearance at such low levels of DEHP has not been reported in other studies, a LOAEL of 0.9 mg/kg bw/day may be an overestimate of risk. Therefore, based on the available studies, it is not possible to motivate the use of this LOAEL for risk characterisation.

Alternatively, the NOAEL for kidney toxicity is considered to be 500 ppm DEHP in the diet (corresponding to 28.9 mg/kg bw/day in the males and 36.1 mg/kg/day in the females) derived from a well-performed 104-week-study in rats (Moore 1996) and based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL: 2,500 ppm corresponding to 146.6 mg/kg bw/day in the males and 181.7 mg/kg bw/day in the females). More severe kidney lesions (increased absolute and relative kidney weights (both sexes), increased incidence and severity of mineralisation of the renal papilla in males, increased incidence and/or severity of tubule cell pigment in both sexes, and increased severity of chronic

progressive nephropathy in the males) were observed at the highest dose level (12,500 ppm corresponding to 789 mg/kg bw/day in the males and 938.5 mg/kg bw/day.

Based on the available data on the DEHP-induced kidney toxicity the NOAEL of 28.9 mg/kg bw/day is selected for risk characterisation, however, the NAEL may be lower if the effects on creatinine clearance at lower doses are substantiated.

In the liver, the most striking effects observed are hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation, and hepatocellular tumours.

Marked species differences are apparent in response to the hepatotoxic effects of DEHP and other peroxisome proliferators (PPs). Rats and mice are very sensitive whereas hamsters, guinea pigs, and monkeys appear to be relatively insensitive or non-responsive at dose levels that produce a marked response in rats. It has been suggested that there may be an association between peroxisome proliferation and the occurrence of liver tumours in rats and mice after long-term exposure. Recent investigations have demonstrated that activation of the peroxisome proliferator-activated receptors (PPAR-α) is required for induction of the different PPs-induced liver effects observed in experimental animals (Peters et al., 1997, Ward et al., 1998 and Cattley et al., 1998). The low sensitivity of human liver to the hepatotoxic effects of PPs could be explained by the low level of PPAR-α found in human liver and genetic variations that render the human PPAR-α less active as compared to PPAR-α in rodent liver (Tugwood et al., 1996, Palmer et al., 1998 and Woodyatt et al., 1999).

Most recently, a Working Group of the "International Agency for Research on Cancer" (IARC) have concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α) is not relevant to humans. Therefor, and based on the overall evaluation of the available data, the DEHP-induced hepatotoxic effects in rats and mice will not be considered in the risk characteriasation.

Other effects have been reported in different studies following repeated dose exposure to DEHP, for example, hypolipidemic effects **(**including decreased plasma levels of cholesterol and triglyceride) and effects on heart, thyroid and lung (including changes in relative and/or absolute weight, and histopathological changes). However, these effects were not considered critical for the risk characteriasation due to the inadequacy of the reported data, lack of reproducibility as well as the severity and/or relevance of the observed effect.

The data available on repeated dose toxicity (not including reproductive effects) do not suggest a classification of DEHP according to EU criteria.

Table 4.30 Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
Rat, Sprague-Dawley females	5 days (2-6, 6-10, or 14-18 of lactation), gavage, corn oil 0 or 2,000 mg/kg/day	↓bw liver: ↑relw all groups, ↑p.enz.act. ↓TG, ↓CHO	Dostal et al. (1987a)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
rat, Sprague-Dawley Males	5 days (from day 6, 14-16, 21, 42, 86 of age) gavage, corn oil 0, 10, 100, 1,000 or 2,000 mg/kg/day comparable to guideline study	mortality: all pups in three youngest age groups at 2,000 mg/kg/day groups ↓bw in two highest dose groups liver: ↑absw and relw from 100 mg/kg/day, ↑pp, ↑p.enz.act. ↓TG, ↓CHO NOAEL 10 mg/kg/day	Dostal et al. (1987b)
Rat, F344 5 males/group	1 week, diet 0 or 1.2% (0 or 670 mg/kg/day**)	liver: ↑absw and relw	Takagi et al. (1992)*
Rat, F344 8 males	1 week, diet 0 or 2% (1,600 mg/kg/day**) comparable to guideline study, GLP	↓CHO, ↓TG ↑ absw and relw for liver and kidney no histological findings in liver, kidney, or testes	Exxon (1982a,b)
Rat, Wistar 4 males/ dose group 6 male controls	3, 10, or 21 days, diet 0 or 2% (1,830, 1,650 or 1,810 mg/kg/day after 3, 10, or 21 days**)	↓bw after 10-21 days liver: ↑relw in all dosed males, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed	Mann et al. (1985)
Rat, Alderley Park 20 rats/sex/group 30 rats/sex in control group	3, 7, 14, 28 days, or 9 months, diet 0, 50, 200 or 1,000 mg/kg/day; comparable to guideline study, GLP	↓bw at 1,000 mg/kg/day for 9 months liver: ↑w from 50 mg/kg/day, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed (males); LOAEL: 50 mg/kg/day	CEFIC (1982) Mitchell et al. (1985a),
Rat, Wistar 6 rats/sex	7 or 21 days, gavage, corn oil 2,500 mg/kg/day	↓bwg (males) liver: ↑relw, no histological findings, ↑nb peroxisomes, ↑pSER	Mangham et al. (1981)*
Rat, F344 4-5 rats/sex/group	1 or 3 weeks, diet 0, 0.1, 0.6, or 1.2% (0, 80, 480, or 960 mg/kg/day**) comparable to guideline study, GLP	liver: ↑w from 0.1%, hepatocellular hypertrophy from 0.6% (males) and at 1.2% (females), ↑nb peroxisomes at 1.2%, ↑p.enz.act. from 0.1% ↓TG from 0.1% ↓CHO from 0.6% LOAEL: 0.1% (80 mg/kg/day)	CMA (1982c)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
Rat, F344 5 rats/sex/group	14 days, diet 0, 6,300, 12,500, 25,000, 50,000 or 100,000 ppm (0, 630, 1,250, 2,500, 5,000 or 10,000 mg/kg/day**)	mortality: 2/5 males and 4/5 females at 100,000 ppm ↓bwg from 25,000 ppm (males), from 50,000 ppm (females) testes: atrophy from 12,500 ppm	NTP (1982)*
Rat, Alderley Park 10 rats/sex/group	14 days, gavage, corn oil 0 or 2,000 mg/kg/day comparable to guideline study, GLP	↓bwg (males) liver: ↑absw, ↑relw, ↑pp, ↑pSER, mitoch changed kidney: ↑weight (females), ↑pp testes: ↓weight, atrophy ↓CHO (males) , ↓TG (males)	ICI (1982b), Rhodes et al. (1986)
Rat, Sprague-Dawley 6 males per group	14 days, gavage, corn oil 0 or 1,000 mg/kg/day	liver: ↑relw, ↑p.enz.act.	Lake et al. (1984a)*
Rat, Sprague-Dawley 5 males per group	14 days, gavage, corn oil 0, 25, 100, 250, or 1,000 mg/kg/day	liver: ↑relw from 100 mg/kg/day, ↑p.enz.act. from 25 mg/kg/day LOAEL 25 mg/kg/day	Lake et al. (1984b)
Rat, Wistar 6 males per group	2 or 4 weeks, diet 0, 60, 200, 600, 2,000 or 6,000 mg/kg (0, 5, 18, 52, 182, or 549 mg/kg/day)	liver: dose-related ↑absw from 2,000 mg/kg following 2 or 4 weeks of treatment, ↑nb peroxisomes from 200 mg/kg, ↑p.enz.act. from 60 mg/kg NOAEL: 60 mg/kg (5 mg/kg bw/day)	RIVM (1992)
Rat, Wistar 5 males per group	14 days, gavage, groundnut oil 0, 250, 500, 1,000 or 2,000 mg/kg/day	↓bw from 1,000 mg/kg/day liver: ↑absw from 1,000 mg/kg/day, ↑relw from 500 mg/kg/day NOAEL 250 mg/kg/day	Khaliq and Srivastava (1993)
Rat, Wistar 5-6 males/group	16 days, diet 0, 0.01, 0.025, 0.05, 0.1, 0.5, or 1.0% (0, 8, 22, 42, 88, 500, or 900 mg/kg/day**)	liver: ↑relw NOAEL: 0.05% (42 mg/kg/day)	Fukuhara and Takabatake (1977)*
Rat, F 344 4-5 males per group	3 weeks, diet 0 or 2%	liver: ↑relw, ↑p.enz.act. hypolipidemia	Moody and Reddy (1978)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
Rat, Sprague-Dawley 4 males	3 weeks, diet 0 or 2% (900 mg/kg/day**)	↓bw and bwg liver: ↑absw and relw, ↑nb peroxisomes, ↑pSER, ↑p.enz.act. kidney: ↑absw and relw ↑CHO and TG (trend only)	General Motors (1982)*
Rat, F344 5 rats/sex/group	21 days, diet 0, 0.01, 0.1, 0.6, 1.2, or 2.5% (0, 11, 105, 667, 1,224 or 2,101 mg/kg/day [males]; 0, 12, 109, 643, 1,197 or 1,892 /kg/day [females]) comparable to guideline study, GLP	↓bw at 2.5%; liver: ↑absw and relw from 0.6%, histological findings from 0.6%, ↑nb peroxisomes from 0.1% (males) and from 0.6% (females), ↑p.enz.act.; kidney: ↑relw at 2.5% testes: ↓w at 2.5%; ↓TG from 0.6% (males) ↑TG at 0.01%(males), 1.2% (females) LOAEL: 0.01% (11 mg/kg/day)	CMA (1984b) Barber et al. (1987)
Rat, F344, 5 males per group	21 days, diet 0, 100, 1,000, 6,000, 12,000 or 25,000 ppm (0, 11, 105, 667, 1,223 or 2,100 mg/kg/day)	liver: ↑relw from 6,000 ppm, ↑nb peroxisomes from 6,000 ppm, ↑p.enz.act. from 1,000 ppm	Short et al. (1987)
Rat, Wistar 3 males/group	4 weeks, diet, 2% or 2 weeks, diet, 2% + 2 weeks control diet	liver: ↑ weight during treatment period, ↓ during withdrawal period; ↑ p.enz.act. during treatment, ↓ during withdrawal	Miyazawa et al. (1980)
Rat, F344 5 rats/sex/group	28 days, diet 0, 0.2, 0.67, or 2.0% (0, 150, 504, or 1563 mg/kg/day [males]; 0, 147, 490 or 1,416 mg/kg/day [females]**) comparable to guideline study, GLP	↓bw at 0.67% liver: ↑absw and relw from 0.2%, ↑p.enz.act. ↓total lipid from 0.2% LOAEL: 0.2% (150 mg/kg/day)	Nuodex (1981c)
Rat, F344 5 males	28 days, gavage, corn oil 1,000 mg/kg/day	liver: ↑absw and relw	Tenneco (1981)*
Rat, F344 5 rats/sex/group	28 days, diet 0 or 0.67% (0 or 350 mg/kg/day**); 21 days, gavage 0 or 700 mg/kg/day	liver: ↑relw, ↑nb peroxisomes, and ↑p.enz.act. at both 0.67% in the diet and 700 mg/kg/day	Hodgson (1987)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
Rat, F344 5 males per dose group 10 male controls	28 days, diet 0, 0.02, 0.05, 0.1, 0.5, 1.0, or 2.5% (0, 24, 52, 115, 559, 1,093 or 2,496 mg/kg/day) GLP	↓bw at 2.5% liver: ↑absw from 0.5%, ↑relw from 0.02%, ↑p.enz.act. from 0.1% testes: ↓absw and relw and atrophy at 2.5% LOAEL 0.02% (24 mg/kg/day)	BIBRA (1990)
Mouse, B6C3F1 10 males per group	5 days, gavage, corn oil 0, 1,879, 2,844, 4,304, 6,514 or 9,860 mg/kg/day GLP	bw and bwg unaffected liver: enlarged with a slight dose-response trend from 1,879 mg/kg/day	Nuodex (1981b)*
Mouse, CD-1 10 females per group	8 days, gavage, corn oil 0, 6,000, 7,690 or 9,860 mg/kg/day GLP	clinical signs of toxicity from 6,000 mg/kg/day; bw and bwg unaffected	Hazleton (1983)*
Mouse, B6C3F1 5 mice/sex/group	14 days, diet 0, 6,300, 12,500, 25,000, 50,000 or 100,000 ppm (0, 630, 1,250, 2,500, 5,000 or 10,000 mg/kg/day**)	mortality: 5/5 (males) and 5/5 (females) at 100,000 ppm, 1/5 (males) and 4/5 (females) at 50,000 ppm ↓bw: from 25,000 ppm (males) and from 50,000 ppm (females) ↓bwg : dose-related	NTP (1982)*
Mouse, B6C3F1 10 mice/sex/group	4 weeks, diet 0, 1,000, 5,000, 10,000 or 25,000 ppm (0, 250, 1,210, 2,580 or 6,990 mg/kg/day** [males]) 0, 270, 1,430, 2,890 or 7,900 mg/kg/day** [females]) US-EPA standard, GLP	mortality: 4/10 (males) and 3/10 (females) at 25,000 ppm ↓bw and bwg from 5,000 ppm (males) and at 25,000 ppm (females) liver: ↑absw and relw from 5,000 ppm, hepatocellular hypertrophy at 25,000 ppm kidney: ↓absw from 5,000 ppm (males) inflammation from 5,000 ppm; testes: ↓absw and relw from 10,000 ppm, atrophy at 25,000 ppm thymus: atrophy at 25,000 ppm ovaries: absence of corpora lutea at 25,000 ppm NOAEL 1,000 ppm (250 mg/kg/day)	Eastman Kodak (1992b)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Short-term studies (up to 28-days exposure)			
F344 rats, 5 males/gr. and B6C3F1 mice, 5 females/gr.	DEHP, 2,000 mg/kg bw, by gavage in corn oil for 14 days. Activities of peroxisome enzymes were assayed in liver homogenates.	Enzymes responsible for production of H_2O_2 peroxisomal palmitoyl CoA oxidase): 9-fold and 21-fold ↑ in rats and mice, respectively. Enzymes responsible for degradation of H_2O_2 Catalase: 2-fold and 3-fold ↑ in rats and mice, respectively. Glutathione peroxidase: ↓ to 50% and 35% of the control in rats and mice respectively.	Tomaszewski et al. (1986)
Subchronic Studies (> 28-days exposure <chronic exposure)			
Rat, Sprague-Dawley 10 rats/sex/group	13 weeks, diet 0, 5, 50, 500 or 5,000 ppm (0, 0.4, 3.7, 37.6, or 375.2 mg/kg/day [males]) 0, 0.4, 4.2, 42.2 or 419.3 mg/kg/day [females]) guideline study, GLP	liver: enlarged (both sexes), ↑absw and relw, hypertrophy, ↑nb peroxisomes at 5,000 ppm kidney: ↑relw at 5,000 ppm (both sexes) thyroid: histological changes at 5,000 ppm testes: mild to moderate Sertoli cell vacuolation from 500 ppm (7/10); ↓ absw and relw testicular weight, atrophy, and complete loss of spermattogenesis at 5,000 ppm (9/10) NOAEL 50 ppm (3.7 mg/kg bw/day)	Poon et al. (1997)
Rat, F344 10 rats/sex/group	13 weeks, diet 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm (0, 80, 160, 320, 630 or 1,250 mg/kg/day**)	↓bwg at 25,000 ppm testes: atrophy from 12,500 ppm NOAEL 6,300 ppm (320 mg/kg/day)	NTP (1982)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Subchronic Studies (> 28-days exposure <chronic exposure)			
Rat, F344 10 rats/sex/group	13 weeks, diet 0, 1,000, 4,000, 12,500 or 25,000 ppm (0, 63, 261, 859, or 1,724 mg/kg/day [males]; 0, 37, 302, 918, 1,858 mg/kg/day [females]) US-EPA standard, GLP	↓bwg from 12,500 ppm (females) and at 25,000 ppm (males) liver: ↑absw and relw from 1,000 ppm, hepatocellular hypertrophy from 4,000 ppm (males) and from 12,500 ppm (females) kidney: ↑relw from 4,000 ppm (males) and from 12,500 ppm (females) testes: ↓w from 12,500 ppm LOAEL: 1,000 ppm (63 mg/kg/day [males], 73 mg/kg/day [females]	Eastman Kodak (1992a)
Mouse, B6C3F1 10 mice/sex/group	13 weeks, diet 0, 800, 1,600, 3,100, 6,300 or 12,500 ppm (0, 100, 200, 400, 800 or 1,600 mg/kg/day**)	↓bwg from 3,100 ppm (males) and from 800 ppm (females) LOAEL 800 ppm 8,100 mg/kg/day)	NTP (1982)
Chronic Toxicity studies (> 10% of the test animals lifespan)			
Rat, Sprague-Dawley 15 rats/sex/group	17 weeks, diet; 0, 0.2, 1.0 or 2.0% (0, 143, 737 or 1,440 mg/kg/day [males]; 0, 154, 797 or 1,414 mg/kg/day [females]) comparable to guideline study	↓bw from 1.0% liver: ↑absw and relw from 0.2%, no histological findings testes: ↓absw and ↑relw from 1.0%, atrophy LOAEL: 0.2% (154 mg/kg/day)	Gray et al. (1977)
Rat, F344 5-10 males per group	1, 2, 4, 8, 18, 39, 77, 151 or 365 days, diet 0 or 1.2% (600 mg/kg/day**)	↑p.enz.act.	Conway et al. (1989)
Rat, Wistar 43 rats/sex/group	3, 6, 12, or 24 months, diet 0, 0.1, or 0.5% (0, 50-80, or 300-400 mg/kg/day**)	overall mortality: 85-96% ↓bw at 0.5% liver and kidney weight increased at 0.5% after 3-6 months but not after 12-24 months no histological findings	Harris et al. (1956)*

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Chronic Toxicity studies (> 10% of the test animals lifespan)			
Rat, Sprague-Dawley 520 males in total	102 weeks, diet 0, 0.02, 0.2 or 2.0% (0, 7, 70 or 700 mg/kg/day**) comparable to guideline study	↓bw from 0.2% liver: ↑pp and nb mitoch from 0.2%, ↑p.enz.act. from 0.02%, no tumours testes: atrophy and inhibition of spermato-genesis from 0.02% LOAEL: 0.02% (7 mg/kg/day)	Ganning et al. (1987, 19901)
Rat, F344 50 rats/sex/group	103 weeks, diet 0, 6,000 or 1,2000 ppm (0, 322 or 674 mg/kg/day [males]; 0, 394 or 774 mg/kg/day [females]) comparable to guideline study, GLP	↓bw at 12,000 ppm hypertrophy anterior pituitary at 12,000 ppm (males); liver: neoplastic lesions from 6,000 ppm; testes: seminiferous tubular degeneration at 6,000 (5%) and at 12,000 ppm (90%)	NTP (1982)
Rat, F344 70-85/sex/group Recovery group: 55/sex	104 weeks, diet 0, 100, 500, 2,500 or 12,500 ppm (0, 5.8, 28.9, 146.6, or 789.0 mg/kg bw/day [males]; 0, 7.3, 36.1, 181.7 or 938.5 mg/kg bw/day [females]) or 12,500 for 78 weeks, followed by a recovery period of 26 weeks	liver: ↑ weight (males) and peroxisome proliferation at 500 ppm; kidney: ↑ weight from 2,500 ppm; ↑ mineralization of the renal papilla (males), tubule cell pigment (both sexes), and chronic progressive nephropathy (males) at 12,500 ppm; pituitary: ↑ castration cells (30/60 males) ar 12,500 ppm testes: ↓ weight, ↑ incidence and severity of bilateral aspermatogenesis; ↓ incidence of interstitial cell neoplasms; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 12,500 ppm; changes in the kidneys, testes, and pituitary were not reversible upon cessation of exposure NOAEL 500 ppm (28.9 mg/kg bw/day [males] and 36.1 mg/kg/day [females])	Moore (1996)
Rat, Sprague-Dawley 5 dosed males, 8 control males	2 years, diet 0 or 2% (0 or 1,000 mg/kg/day**)	liver: ↑relw, ↑ nb mitoch ↑nb peroxisome ↑p.enz.act. and lipid peroxidation	Lake et al. (1987)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Chronic Toxicity studies (> 10% of the test animals lifespan)			
Rat, Sprague-Dawley 10 rats/sex/group	Life-time, diet 0 or 0.5% (0 or 200 mg/kg/day)	liver: necroses and fat infiltration in a few animals kidney: nephroses in a few animals	BASF (1960)*
Mouse, B6C3F1 50 mice/sex/group	103 weeks, diet 0, 3,000 or 6,000 ppm (0, 672 or 1,325 mg/kg/day (males) 0, 799, or 1,821 mg/kg/day (females) GLP	↓bw at 6,000 ppm (females) liver: hepatocellular neoplastic lesions kidney: inflammation at 6,000 ppm (males) testes: seminiferous tubular degeneration and testicular atrophy at 6,000 ppm	NTP (1982)
Mouse, B6C3F1 70-85/sex/group; Recovery group: 55/sex	104 weeks, diet 0, 100, 500, 1,500 or 6,000 ppm (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg/day [males]; (0, 23.8, 116.8, 354.2 or 1,458.2 mg/kg/day [females]) or 6,000 ppm followed by a recovery period of 26 weeks	liver: peroxisome proliferation and ↑ weight (males) from 500 ppm; ↑ weight, adenomas and carcinomas (both sexes) from 1,500 ppm; kidney: ↓ weight (especially males) and chronic progressive nephropathy (both sexes) from 1,500 ppm; testes: ↓ weight, ↑ incidence and severity of bilateral hypospermia from 1,500 ppm; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 1,500 ppm; ↓ survival (males) changes in liver, kidneys, and testes were at least partially reversible following recovery period; NOAEL 100 ppm (19.2 mg/kg [males] and 23.8 mg/kg [females])	Moore (1997)

Table 4.30 continued overleaf

Table 4.30 continued Effects of DEHP following repeated oral administration

Species	Protocol	Results	References
Chronic Toxicity studies (> 10% of the test animals lifespan)			
Male rats, 4 rats from each dose group were sacrificed at 3, 6, 9, and 12 months.	One year, by gavage, three times weekly, 3 dose groups: (a) 150 mg DEHP/70 kg bw, approximately 0.9 mg/kg bw/day, (b) 150 mg leachate (leachate resulting from toluene extraction of an phthalates-containing artificial kidney)/70 kg bw, approximately 0.9 mg/kg bw/day (c) sesame oil controls	statistically significant increase (p= 0.04) in the incidence of **focal cystic changes** was observed in kidneys of rats received DEHP or leachate and killed at 12-month interval. **Creatinine clearance** was significantly decreased (p = < 0.01) only in rats receaved pure DEHP and killed at the 12-month interval.	Crocker et al., 1988

↓ / ↑ Decreased and increased, respectively
bw / bwg Body weight and body weight gain, respectively
absw / relw Absolute and relative weight, respectively
mitoch Mitochondria
nb Number
p.enz.act. Peroxisomal enzyme activity or activities;
pp Peroxisome proliferation
pSER Proliferation of smooth endoplasmatic reticulum
CHO / TG Cholesterol and triglyceride, respectively
w Weight
* Studies not included in the risk assessment
** The conversion of daily intakes into mg/kg bw/day have been derived from IUCLID
NOAEL-values for the risk assessment have been selected from studies with references in extra **bold** type

Table 4.31 Summary of some *in vitro* studies on peroxisome proliferative activity

Species	Protocol	Results	References
In vitro, hepatocytes from Wistar rats	MEHP, 200 or 300 μM (57 or 84 μg/ml) , Gap Junctional Intercellular Communication (GJIC) was studied.	CJIC was inhibited, Authors Concl.: inhibition of GJIC may contribute to the tumour promoting activity of MEHP, but is not sufficient for their tumour promoting action	Leibold et al. (1994)
In vitro, human hepatocytes	MEHP, 0.2 mM (56 μg/ml) for 48 or 72 hours	No induction of the enzymes palmitoyl-CoA oxidase (a peroxisome specific enzyme) and carnitine acetyltransferase (a peroxisomal enzyme also found in mitochondria)	Butterworth et al. (1989)
In vitro, nontumouro genic line of mouse liver epithelial cells, BNL-CL.2	DEHP, 0.1 or 1 mM (39 or 390 μg/ ml) for 1 or 4 hours. The effect on fos and jun family of nuclear proto-oncogenes was investigated.	DEHP (1mM) caused a very small ↑ in c-fos and c-jun expression, but the substance was a fairly strong inducer of jun-B and jun-D expression. Authors Concl.: DEHP activates growth-regulatory signal transduction pathways that lead to the immediate-early induction of the *fos* and *jun* family of nuclear proto-oncogenes. This gene activation must be independent of the effects on peroxisome proliferation, since the BNL-CL.2 cell line does not undergo peroxisome proliferation and since the induction of immediate-early gene expression would precede peroxisome proliferation in cells that do undergo peroxisome proliferation.	Ledwith et al. (1993)

↓/↑ Decreased/increased, respectively

4.1.2.8 Mutagenicity

The possible genotoxic effect of DEHP has been thoroughly investigated in several different short-term tests. A great number of these studies were part in the International Programme on Chemical Safety's (IPCS) collaborative study on evaluation of short-term tests for carcinogens (Ashby et al., 1985). The major metabolites of DEHP, MEHP and 2-EH, have also been examined and the results of these studies will appear in the text.

Few studies have been conducted according to Annex V, however, a number of studies are comparable to guideline studies. If studies are performed according to a guideline and to GLP principles, or are comparable to guideline studies, it will be obvious in the text. When expressing results, the term "significant" is used only if the result is statistically significant at a p-level lower than 0.05.

4.1.2.8.1 *In vitro* studies

Gene mutation

(see **Table 4.32**).

Bacteria

DEHP and MEHP were tested in *Salmonella typhimurium* (TA98, TA100) and *Escherichia coli* trp^- (uvr^+, uvr^-) at concentrations from 50 to 2,000 µg/plate with and without metabolic activation with rat S9-mix (Yoshikawa et al., 1983). DEHP and MEHP did not show any mutagenic activity but some lethality towards the *S. typhimurium* strains.

In a study comparable to a guideline study and performed according to GLP principles, DEHP, MEHP and 2-EH were tested in *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) at concentrations from 0.1-10 µl DEHP/plate (purity: 99.9%) (98-9,800 µg/plate), 0.002-0.2 µl MEHP/plate (purity: 94.7%), and 0.01-1.0 µl 2-EH/plate (purity: 99.7%) (8.3-830 µg/plate) with and without metabolic activation with Arochlor-induced rat liver S9 (Nuodex, 1980 and Kirby et al., 1983). No significant mutagenic activity was observed for any of the substances.

In a study comparable to a guideline study and performed according to GLP principles, DEHP and MEHP were tested in the standard Ames test and 2-EH was tested in a volatile containment system using *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) at concentrations from 0.5 to 5,000 µg DEHP/plate (purity: > 97%), 7.5-750 µg MEHP/plate (purity: > 97%), and 0.4-1.8 µg 2-EH/vessel (purity: > 97%) with and without metabolic activation with rat S9-mix (Eastman Kodak, 1984b and DiVincenzo et al., 1985). No increase in revertants was observed in any of the tester strains neither with nor without metabolic activation. In a second test, the mutagenicity of urine from rats treated with DEHP or 2-EH was examined. Male Sprague-Dawley rats were administered a daily dose of 2,000 mg/kg bw of DEHP or 1,000 mg/kg bw of 2-EH in corn oil by gavage for 15 days. Urine was collected and pooled and tested undiluted by a direct plating procedure (0.02, 0.06, 0.20, 0.66, 2.0 ml urine per plate) in *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) with and without metabolic activation with rat S9-mix. There was no evidence that mutagenic substances were excreted in the urine following administration of DEHP or 2-EH.

In a study comparable to a guideline study, DEHP, MEHP and 2-EH were tested in *S.typhimurium* (TA98, TA100, TA1535, TA1537) with quantities of 100-10,000 µg DEHP/plate (purity: 99.6%), 10-3,333 µg MEHP/plate (purity: not stated), and 3.3-220 µg 2-EH/plate

(purity: 99%) with and without metabolic activation with S9-mix from rat and hamster (Zeiger et al., 1982, 1985a, 1985b). DEHP, MEHP and 2-EH exhibited no mutagenicity in any of the tester strains neither with nor without metabolic activation.

DEHP (purity: > 99%) was tested in (TA102), a strain sensitive to mutations arising as a cause of oxidative DNA damage, in concentrations from 1.0 to 20.0 µmol/plate (391-7,812 µg/plate) in the presence of enzymes proposed to be responsible for the metabolic activation of DEHP (Schmezer et al., 1988). No mutagenic response was observed. Similarly, MEHP (purity not stated) was not mutagenic in *S. typhimurium* (TA100, TA102) when tested in concentrations from 0.16 to 1.25 µmol/plate (45-348 µg/plate) with and without metabolic activation with S9 (rat).

DEHP (purity: analytical grade) and MEHP (purity: > 99%) were tested in *S. typhimurium* (TA100) at a concentration of 5,000 µg/plate in the presence of rat liver S9-mix (DEHP) or at a concentration of 1250 µg/plate with and without metabolic activation (MEHP) (Tomita et al., 1982a). According to the authors, the results for DEHP and MEHP were significantly different from controls. However, DEHP or MEHP did not induce a doubling of the number of revertants/plate compared to control, therefore the positive result may be considered as being only marginally positive.

Four metabolites of DEHP (MEHP, metabolites V, VI, IX) were tested for mutagenicity in the Ames assay using *S. typhimurium* (TA97, TA98, TA100, TA102) at concentrations of 0, 0.5, 2, 10, 50, 200, 1,000 µg/plate with and without metabolic activation with Arochlor 1254-induced rat liver S9 or DEHP-induced rat liver S9 (Dirven et al., 1991). Negative results were obtained for all four metabolites in the absence and presence of metabolic activation.

Fungi

DEHP (purity: not stated) was tested in *Saccharomyces cerevisiae* for point mutations in strains XV185-14C and RM52 using two different cell culture conditions at concentrations from 1,541 to 12,325 nl/ml (1,510-12,080 µg/ml) with and without metabolic activation with rat S9 (Mehta and van Borstel, 1985). DEHP induced point mutations his^+ with S9 in strain XV185-14C cultured in buffer pH 7.0 being significant only in the lowest and the highest concentration. When cultured in YEPD medium pH 6.3, DEHP induced point mutations his^+ in strain RM52 (-S9), arg^+/his^+ (+/-S9), trp^+ (+S9) in strain XV185-14C. Mutation frequencies were 2-fold increased but not dose-dependent. According to the authors, overall test results were positive.

DEHP was tested in the *ade* forward-mutation system in *Schizosaccharomyces pombe* P1 in concentrations from 369 to 5,870 µg/ml with and without metabolic activation with S9 (rat) (Loprieno et al., 1985). According to the authors, 3-fold increases in mutant frequency were obtained over 3 consecutive doses in the assay without metabolic activation, but in a second experiment this finding was not confirmed. The results were therefore regarded as equivocal.

Mammalian cells

DEHP, MEHP and 2-EH were tested in the L5178Y $TK^+/-$ mouse lymphoma cell mutagenicity assay with and without metabolic activation with S9 (Nuodex, 1981d and Kirby et al., 1983). DEHP (purity: 99.9%) was tested at concentrations from 0.016 to 1.0 µl/ml (15-206 µg/ml) without activation and from 0.067- 5.0 µl/ml (66-4,900 µg/ml) with activation without exhibiting any significant mutagenic activity. Unlike DEHP, in the initial toxicity test both MEHP (purity: 94.7%) and 2-EH (purity: 99.7%) demonstrated complete toxicity at concentrations > 1.0 µl/ml.

These metabolites were therefore tested at concentrations from 0.013 to 1 µl/ml, the results obtained were negative.

DEHP was tested for the ability to induce mammalian cell mutation in the L5178Y $TK^+/-$ mouse lymphoma cell forward-mutation assay with and without metabolic activation with rat S9 at concentrations from 10 to 620 µg/ml (-S9) or 1.0-80 µg/ml (+S9) (Oberly et al., 1985). A dose-dependent decrease in values for total survival was obtained in the absence of S9 ranging from 5 to 73% and a 2-fold or greater increase in mutation frequency was seen at 2 dose levels, however, survival was only 12 and 5% at these dose levels. In the presence of S9, a dose-related decrease in total survival was observed ranging from 5 to 90% and a 2-fold or greater increase in mutant frequency was seen at three dose levels. According to the authors, DEHP was mutagenic in the assay with metabolic activation.

Table 4.32 Gene mutation *in vitro*

Species	Protocol	Results	Reference
Bacteria			
S. typhimurium TA98, TA100	Ames test 50, 100, 200, 500, 1,000, 2,000 µg/plate; -/+ S9-mix (rat)	negative	Yoshikawa et al. (1983)
S. typhimurium TA98, TA100,TA1535, TA1537, TA1538	Ames test 50, 100, 500, 1,000, 5,000 µg/plate, -/+ S9 (rat)	negative	Rexroat and Probst (1985)*
S. typhimurium TA98, TA100,TA1535, TA1537, TA1538	Ames test 0.1, 0.5, 2.5, 5.0, 10.0 µl/plate (98-9,800 µg/plate); -/+ Arochlor-induced rat liver S9; comparable to guideline study, GLP	negative	Nuodex (1980), Kirby et al. (1983)
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test 0.5, 5.0, 50, 500, 5,000 µg/plate, -/+ S9-mix (rat); comparable to guideline study, GLP	negative	Eastman Kodak (1984b), DiVincenzo et al. (1985)
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test 0.15, 0.47, 1.50, 4.74, 15.0, 47.43, 150.0 µl/plate (147-14,700 µg/plate), -/+ Arochlor-induced rat liver S9; comparable to guideline study, GLP	negative	CMA (1982d)*
S. typhimurium TA98, TA100,TA1535, TA1537, TA1538, TA2637	spot-test 500 µg/plate	negative	Agarwal et al*. (1985a)
S. typhimurium TA98, TA100, TA1535, TA1537	Ames test 100-2,000 µg/plate -/+ S9 (rat)	negative	Agarwal et al.* (1985a)
S. typhimurium TA98, TA100, TA1535, TA1537	Ames test 100, 333, 1,000, 3,333, 10,000 µg/plate; -/+ S9-mix (rat, hamster); comparable to guideline study	negative	Zeiger et al. (1982, 1985a, 1985b)

Table 4.32 continued overleaf

Table 4.32 continued Gene mutation *in vitro*

Species	Protocol	Results	Reference
Bacteria			
S. typhimurium TA98, TA100	Ames test up to 1,000 µg/plate; -/+ S9 (rat)	negative	Kozumbo et al. (1982)*
S. typhimurium TA102	Ames test 0, 1.0, 2.5, 5.0, 10.0, 20.0 µmol/plate (391-7,812 µg/plate), various enzymes	negative	Schmezer et al. (1988)
S. typhimurium TA102	Ames test up to 5,000 µg/plate; -/+ S9-mix (rat); comparable to guideline study	negative	Jung et al. (1992)*
S. typhimurium TA97, TA98, TA100, TA102	Ames test 0, 320, 1,000, 3,200, 10,000 µg/plate, -/+ S9-mix (rat)	negative	Baker and Bonin (1985)*
S. typhimurium TA100	Ames test 5 mg/plate, + S9-mix (rat)	marginally positive, see text	Tomita et al. (1982a)
S. typhimurium TA97, TA98, TA100, TA102	Ames test 0, 100, 200, 500, 1,000, 2,000, 5,000 µg/plate; -/+ S9 (rat)	negative	Matsushima et al. (1985)*
S. typhimurium TA98, TA100	Ames test 0.30, 39, 3,900 µg/ml; -/+ S9 (rat)	negative	Warren et al. (1982)*
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test 0.02, 0.06, 0.20, 0.66, 2.00 ml urine from rats treated 15 days with 2,000 mg/kg/day; -/+ S9-mix (rat)	negative	Eastman Kodak (1984b), DiVincenzo et al. (1985)
S. typhimurium TM677	bacterial forward mutation assay 50, 200, 500 µg/ml; -/+ S9 (rat)	negative	Liber (1985)*
E.coli *trp*$^-$ (*uvr*$^+$, *uvr*$^-$)	Ames test 50, 100, 200, 500, 1,000, 2,000 µg/plate, -/+ S9 (rat)	negative	Yoshikawa et al. (1983)
Fungi			
S. cerevisiae PV-1, PV-2, PV-3	gene mutation 1-1,000 µg/ml -/+ S9 (rat)	negative forward mutation (PV-1) and reverse mutation (PV-2, PV-3)	Inge-Vechtomov et al. (1985)*
S. cerevisiae D7	gene mutation 0, 40, 200, 1,000, 5,000 µg/ml, -/+ S9	negative point mutation	Arni (1985)*
S. cerevisiae D7	gene mutation up to 50 mg/ml	negative point mutation	Parry (1985)*

Table 4.32 continued overleaf

Table 4.32 continued Gene mutation *in vitro*

Species	Protocol	Results	Reference
Fungi			
S. cerevisiae D7	gene mutation 200, 500, 1,000, 2,000, 3,000, 5,000 µg/ml; -/+ S9-mix (rat)	negative point mutation	Parry and Eckardt (1985)*
S. cerevisiae XV185-14C, RM52	gene mutation 1,541, 3,081, 6,163, 12,325 nl/ml (1,510-12,080 µg/ml); -/+ S9 (rat)	equivocal, see text	Mehta and van Borstel (1985)
S. pombe P1	forward mutation assay 369, 738, 1,467, 2,935, ,5870 µg/ml, -/+ S9(rat)	equivocal, see text	Loprieno et al. (1985)
Mammalian cells			
Chinese hamster ovary cells CHO-K1-BH4	forward mutation (HGPRT) 5, 10, 20, 40, 80 nl/ml (4.9-78 µg/ml), -/+ S9 (rat); comparable to guideline study, GLP	negative for mutant frequency	CMA (1985)*
Mouse embryo cells Balb/c-3T3	gene mutation 0, 79, 250, 791, 2,000, 7,910 nl/ml (77-7,752 µg/ml); + S9 (rat)	negative for ouabain resistance	Matthews et al. (1985)*
Mouse lymphoma cells L5178Y	gene mutation (fluctuation assay) 12.5-200 µg/ml, -/+ S9 (rat)	negative for ouabain and 6-thioguanine resistance	Garner and Campbell (1985)*
Mouse lymphoma cells L5178Y TK^{+}/-	gene mutation 184-2,468 µg/ml +S9 22-301 µg/ml -S9	negative for trifluoro-thymidine resistance with S9; inconclusive without S9	Amacher and Turner (1985)*
Mouse lymphoma cells L5178Y TK^{+}/-, L5178Y clone 372+/+	mouse lymphoma assay 0, 78, 392, 1,962, 9,810 µg/ml, -/+S9 (rat)	negative	Styles et al. (1985)*
Mouse lymphoma cells L5178Y TK^{+}/-	mouse lymphoma assay 0.016-1.0 µl/ml (15-206 µg/ml); -S9 (rat); 0.067-5.0 µl/ml (66-4,900 µg/ml) +S9 (rat); compar-able to guideline study, GLP	negative	Nuodex (1981d), Kirby et al. (1983)
Mouse lymphoma cells L5178Y TK^{+}/-	Mouse lymphoma assay, 10, 20, 30, 40, 50, 100, 200, 400, 620 µg/ml -S9 (rat); 1.0, 2.5, 5.0, 7.5, 10, 20, 40, 80 µg/ml +S9 (rat)	positive	Oberly et al. (1985)
Mouse lymphoma cells L5178Y TK^{+}/-	Mouse lymphoma assay 250, 500, 1,000, 2,000, 3,000, 5,000 nl/ml (245-4,900 µg/ml), -/+S9(rat)	negative	Myhr et al. (1985)*

Table 4.32 continued overleaf

Table 4.32 continued Gene mutation *in vitro*

Species	Protocol	Results	Reference
Mammalian cells			
Human lymphoblasts TK6, AHH-1	gene mutation 0, 200, 250, 400, 600, 750, 800, 1,000 µg/ml; -/+S9 (rat) (TK6) -S9 (AHH-1)	negative for gene-locus mutations	Crespi et al. (1985)*

* Studies which are only included in the table;
E.coli *Escherichia coli*;
S. cerevisiae *Saccharomyces cerevisiae*;
S. typhimurium *Salmonella typhimurium*;
-/+ S9 Without/with metabolic activation with S9 liver-tissue fraction from rat or hamster
DNA damage (see Table 4.33)

Bacteria

The DNA damaging potential of DEHP, MEHP and 2-EH was tested in the Rec-assay with *Bacillus subtilis* (H-17 (rec^+), M-45 (rec^-) (Tomita et al., 1982a). DEHP (purity: analytical grade) was negative when tested in a concentration of 500 µg/plate. MEHP (purity: > 99%) was positive when tested in concentrations of 400 and 500 µg/plate but negative in concentrations ranging from 50 to 300 µg/plate. 2-EH (purity: analytical grade) was slightly positive at a concentration of 500 µg/plate (see **Table 4.33**).

Mammalian cells

DEHP and MEHP were tested for induction of unscheduled DNA synthesis (UDS) in primary mouse hepatocytes at concentrations from 0.01 to 1.0 mM DEHP (purity: 99.8%) (3.9-390 µg/ml) or 0.1-0.5 mM MEHP (purity: not stated) (28-139 µg/ml) with negative results (Smith-Oliver and Butterworth, 1987).

DEHP (purity: not stated) was tested for the capacity to enhance the adenovirus (SA7) transformation of Syrian hamster embryo cells (SHE) at concentrations from 0, 0.2, 0.3, 0.6, 1.3 and 2.6 mM (78-1,016 µg/ml) (Hatch and Anderson, 1985). No enhancement of virus transformation was observed in a first experiment. A second experiment detected activity at the two highest concentrations that was independent of cytotoxicity for significance.

Table 4.33 DNA damage *in vitro*

Species	Protocol	Results	Reference
Bacillus subtilis H17 (rec^+), M45 (rec^-)	Recombination assay 500 µg/plate	negative	Tomita et al. (1982a)
rat hepatocytes	DNA damage and repair assay 391, 1,172, 3,907 µg/ml	negative for single-strand breaks	Bradley (1985)*
rat and hamster hepatocytes	DNA damage and repair assay 0, 1.25, 2.5, 3.125, 5.0, 12.5, 25.0 µmol/tube (488-9765 µg/tube)	negative for single-strand breaks	Schmezer et al. (1988)*
Chinese hamster ovary cells (CHO)	DNA damage and repair assay 391, 1,170, 1,950, 2,730, 3,910 µg/ml	negative for single-strand breaks	Douglas et al. (1985, 1986)*

Table 4.33 continued overleaf

Table 4.33 continued DNA damage *in vitro*

Species	Protocol	Results	Reference
Syrian hamster embryo cells (SHE)	DNA damage and repair assay (DNA adenovirus (SA7) trans-formation); 0, 0.2, 0.3, 0.6, 1.3, 2.6 mM (78-1,016 µg/ml)	equivocal, see text	Hatch and Anderson (1985)
Primary rat hepatocytes	UDS 0.19, 0.39, 1.95, 3.9, 19.5, 39, 195, 390, 1,950, 3,900 µg/ml	negative	Probst and Hill (1985)*
Primary rat hepatocytes and primary human hepatocytes	UDS DNA repair assay 0.1, 1, 10 mM (39-3,900 µg/ml) comparable to guideline study	negative	Butterworth et al. (1989, 1984)*
Primary rat hepatocytes	UDS, DNA repair assay 0.01, 0.1, 1, 10 mM (3.9-3,900 µg/ml) comparable to guideline study	negative	Kornbrust et al. (1984)*
Primary rat hepatocytes	UDS 0.1, 1, 10, 100, 1,000, 10,000 µg/ml	negative	Williams et al. (1985)*
Primary rat hepatocytes	UDS 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5.0, 10.0 µl/ml (76-9,800 µg/ml); comparable to guideline study, GLP	negative	Nuodex (1981e)*
Primary mouse hepatocytes	UDS, DNA repair assay 0.01, 0.1, 1 mM (3.9-390 µg/ml)	negative	Smith-Oliver and Butterworth (1987)

* Studies which are only included in the table;
UDS Unscheduled DNA synthesis

Chromosomal effects

(See **Table 4.34**).

DEHP (purity: not stated) was tested in a fibroblast cell line (CH1-L) from Chinese hamster at concentrations up to 50 µl/ml (49 mg/ml) without metabolic activation (Parry et al., 1984). DEHP produced increases in hyperploidy cells (chromosome number > 22) but was negative for hypoploidy and polyploidy cells.

DEHP (purity: not stated) was tested in primary liver cells (CH1-L) from Chinese hamster at concentrations from 5 to 50 mg/ml (Parry, 1985). The mitotic index was reduced at the top dose, the AT/M ratio was reduced in a dose related manner, and the chromosome cluster group and the abnormal division stage was increased by treatment. According to the author, these observations indicate a positive spindle effect. There was no effect on chromosome dislocation, multipolar spindles, or chromosome lagging and bridge formation.

DEHP, MEHP and 2-EH were tested for clastogenic activity in cultured Chinese hamster ovary (CHO) cells (Phillips et al., 1982). DEHP (purity: 99%) did not induce chromosome aberration (CA) at concentrations from 0.5 to 2.0 mM (195-780 µg/ml), 2-EH (purity: not stated) had a slight effect at the highest dose level tested (2.4 mM (313 µg/ml)). MEHP (purity: not stated) had a marked effect on chromosome integrity at doses in the cytotoxic range. Concentrations of MEHP up to 1.25 mM (348 µg/ml) caused increasing amounts of chromosome damage but above 1.25 mM no further increase was observed. MEHP was without effect in the tests for sister-chromatid exchange and for HGPRT cell-mutation.

DEHP and MEHP were tested in cultured Syrian hamster embryo (SHE) cells at concentrations from 1 to 100 µM DEHP (purity: not stated) (0.39-39 µg/ml) or 1-300 µM MEHP (purity: not stated) (0.28-84 µg/ml) with and without metabolic activation with rat S9 (Tsutsui et al., 1993). Chromosome aberrations were induced following exposure of cells for 2 hours to DEHP and MEHP in the presence of metabolic activation, but were not induced by treatment for 24 hours with DEHP and MEHP in the absence of metabolic activation.

Table 4.34 Chromosomal effects *in vitro*

Species	Protocol	Results	Reference
S. cerevisiae D7	induction of mitotic aneuploidy up to 50 µl/ml (49 mg/ml)	positive increase in hyperploidy (chromosome no >22)	Parry (1985)*
S. cerevisiae D61.M	induction of mitotic aneuploidy saturated solution	negative	Zimmerman et al. (1985)*
Chinese hamster liver cells, CH1-L	cytogenetic assay up to 50 µl/ml (49 mg/ml)	positive increase in hyperploidy (chromosome no >22)	Parry et al. (1984)
Chinese hamster liver cells, CH1-L	cytogenetic assay 5, 12.5, 25, 50 mg/ml	positive effect on the spindle	Parry (1985)
human leukocytes	cytogenetic assay 0.06, 0.6, 6, 60 µg/ml	negative for breaks, gaps, abnormal forms	Stenchever et al. (1976)*
human foetal lung cells	cytogenetic assay 6 µg/ml	negative for breaks, gaps, abnormal forms, aneuploidy	Stenchever et al. (1976)*
rat liver cells RL4	cytogenetic assay 0, 125, 250, 500, 1,000 µg/ml	negative for CA and SCE	Priston and Dean (1985)*, Shell (1983)*
Chinese hamster lung fibroblasts (CHL)	cytogenetic assay 1,375, 2,750, 4,130 µg/ml; -/+ S9 (rat)	negative for CA	Ishidate and Sofuni (1985)*
Chinese hamster ovary cells (CHO)	cytogenetic assay 0.5, 1.0, 2.0 mM (195-780 µg/ml)	negative for CA	Phillips et al. (1982)
Chinese hamster ovary cells (CHO)	cytogenetic assay 50, 160, 500, 1,600, 2,000, 3,000, 4,000, 5,000 µg/ml (CA), -/+ S9(rat); 5, 16, 50, 160, 500, 1,600, 3,000, 4,000, 5,000 µg/ml (SCE); -/+ S9(rat). comparable to guideline study	negative for CA and SCE	Gulati et al. (1985)*, Gulati et al. (1989)*

Table 4.34 continued overleaf

Table 4.34 continued Chromosomal effects *in vitro*

Species	Protocol	Results	Reference
Chinese hamster ovary cells (CHO)	cytogenetic assay 3.9, 19.5, 39, 195, 390, 1,170, 2,340, 3,900 µg/ml; -/+ S9 (rat)	negative for SCE	Douglas et al. (1985, 1986)*
Chinese hamster ovary cells (CHO)	micronucleus assay 0.1, 1, 10, 100 mM (3.9-3,900 µg/ml), -/+ S9 (rat)	negative	Douglas et al. (1985, 1986)*
human lymphocytes	cytogenetic assay 3.2, 15.7, 30.6, 45.0, 61.3, 75.4 µg/ml	negative for CA	Turner et al. (1974)*
Syrian hamster embryo cells (SHE)	cytogenetic assay 0, 1, 3, 10, 30, 100 µM (0.39-39 µg/ml); -/+ S9 (rat)	positive for CA	Tsutsui et al. (1993)
human peripheral lymphocytes	cytogenetic assay 10, 100, 1,000 µg/ml -/+ S9 (rat)	negative for SCE	Obe et al. (1985)*

* Studies which are only included in the table;
CA Chromosome aberration;
SCE Sister-chromatid exchange;
S. cerevisiae *Saccharomyces cerevisiae*

4.1.2.8.2 *In vivo* studies

Gene mutation: Studies on gene mutation *in vivo* are summarised in **Table 4.35**. Abbreviations are explained below the table.

Table 4.35 Gene mutation in vivo

Species	Protocol	Results	References
mouse, C57BL/6f lacI transgenic	transgenic mouse mutation assay 120 days, diet, 3,000, 6,000 ppm (600, 1,200 mg/kg/d**)	negative no increase in mutant frequency in the lacI gene of liver DNA	Gunz et al. (1993)
Drosophila melanogaster	somatic mutation assay (unstable eye mosaic test) 96 hours, addition to culture medium of 10, 20, 40, 80, 160, 320 mM (3.9-125 mg/ml)	positive at 20 mM negative at other concentrations	Fujikawa et al. (1985)
Drosophila melanogaster	somatic mutation assay 4 days, feed 2 mM (0.78 mg/ml)	questionable results	Vogel (1985)
Drosophila melanogaster	somatic mutation assay (wing spot tests) 48, 72, 96 hours, feed 200 mM (78 mg/ml)	Negative except for induction of twin spots after 48 hours	Würgler et al. (1985)

Table 4.35 continued overleaf

Table 4.35 continued Gene mutation *in vivo*

Species	Protocol	Results	References
Drosophila melanogaster	somatic mutation assay (wing spot tests) 48 hours, feed 200 mM (78 mg/ml)	positive for large single spots; ambiguous for twin spots; negative for small single spots	Graf et al. (1989)

** quoted from IUCLID;
mg/kg/d mg/kg bw/day

DNA damage

(see **Table 4.36**).

To evaluate the relationship between hydrogen peroxide generation and subsequent DNA damage caused by peroxisome proliferation, DNA damage and changes in peroxisomal ß-oxidation activity in rat liver were examined (Tamura et al., 1991). In male F-344 rats (4 rats/group) fed 2% DEHP in the diet for 52 or 78 weeks, hepatocarcinomas or neoplastic nodules were seen in 1/4 or 2/4 rats, respectively. The hepatic DNA from tumour-bearing rats showed a 5-fold increase in single strand DNA breaks whereas no increase was observed in non tumour-bearing rats. According to the authors, the results show that although prolonged treatment with peroxisome proliferators induces markedly peroxisomal ß-oxidation activity, the active oxygen species from peroxisomal ß-oxidation are not enough to give rise to significant DNA damage. On the other hand, Nilsson et al. (1991) found no evidence of DNA damage induced by the potent peroxisome proliferator nafenopin *in vivo*, either measured as single strand breaks in presence of inhibitors of DNA-repair, or as effects on unscheduled DNA-synthesis. Similar results were obtained even when oxidative stress was induced by administration of buthionine sulfoximine at a dose that drastically lowered the endogenous glutathione pool in liver. Nafenopin also failed to potentiate the yield of micronuclei in hepatocytes induced by ionising radation (Nilsson et al., 1991).

Table 4.36 DNA damage *in vivo*

Species	Protocol	Results	Reference
Rat, F344 3 males per group	DNA-adduct formation 3 days, gavage 2,000 mg/kg/d	^{32}P-postlabelling of hepatic DNA preparations revealed that no adducts were detected	Gupta et al. (1985)*
Rat, F344 Females	DNA-binding study single dose of 500 mg/kg carbonyl-^{14}C or alcohol-^{14}C labelled; with or without a 4-week prefeeding period with 1% in the diet	carbonyl-^{14}C: no radioactivity was detectable in liver DNA alcohol-^{14}C: radioactivity was clearly measurable in the DNA	BASF (1982)*
Rat, F344 3 males per group	DNA-damage 1, 2, 3, 6, 9, 12 months, diet 1.2% (600 mg/kg/d**)	levels of 8-OH-deoxyguanosine (marker for oxidative DNA-damage) in liver DNA was 2-fold increased after 1 month	Takagi et al. (1990)*

Table 4.36 continued overleaf

Table 4.36 continued DNA damage *in vivo*

Species	Protocol	Results	Reference
Rat, F344 4 males per group	DNA-damage 2, 40, 78 weeks, diet 2% (900 mg/kg/d**)	5-fold increase in single strand breaks in tumour-bearing rats; no increase in other rats	Tamura et al. (1991)
Rat, F344 Males	UDS, DNA repair assay, alkaline elution assay 14 days, gavage in corn oil, 150 mg/kg/d; 500 mg/kg 2, 12, 24, 48 hours before sacrifice	negative DNA repair and DNA damage (strand breakage) was not induced in hepatocytes isolated from treated rats	Butterworth et al. (1984)*
Rat, F344 Males	UDS, DNA repair assay, alkaline elution assay 30 days, feed 12,000 ppm (600 mg/kg/d**); followed by 500 mg/kg by gavage	negative DNA repair and DNA damage (strand breakage) was not induced in hepatocytes isolated from treated rats	Butterworth et al. (1984)*
Rat, Sprague-Dawley Males	UDS, DNA repair assay 4 or 8 weeks, gavage 5,000 mg/kg; 4 or 8 weeks, feed 2% (1,000 mg/kg/d**); followed by 5,000 mg/kg by gavage	negative DNA repair was not induced in hepatocytes isolated from treated rats	Kornbrust et al. (1984)*
Mouse, B6C3F1 Males	UDS, DNA repair assay 7, 14, 28 days, gavage in corn oil 10, 100, 500 mg/kg; 7, 14, 28 days, feed 6,000 ppm (1,200 mg/kg/d**)	negative DNA repair was not induced in hepatocytes isolated from treated mice	Smith-Oliver and Butterworth (1987)*

* Studies which are only included in the table;
** Quoted from IUCLID;
mg/kg/d mg/kg bw/day;
CA Chromosomal aberration;
Sc Subcutane,
UDS Unscheduled DNA synthesis

Chromosomal effects

(see **Table 4.37**).

In a study performed according to GLP principles, DEHP, MEHP and 2-EH were tested for their ability to induce chromosomal damage in male Fischer rats after oral administration (Putman et al., 1983 and Nuodex, 1981i). Five rats per group were given by gavage in corn oil 0.5, 1.7, 5.0 ml/kg/day of DEHP (purity: 99.9%), 0.01, 0.05, 0.14 ml/kg/day of MEHP (purity: 94.7%), or 0.02, 0.07, 0.21 ml/kg/day of 2-EH (purity: 99.7%) for 5 consecutive days. A positive control group was included. No significant increase in chromatid and chromosome breaks or structural rearrangements were noted and the mitotic index was also unaffected.

Chromosomal aberrations and morphological transformations were assayed in Syrian golden hamster (SHE) embryo cells after transplacental administration of DEHP or MEHP (Tomita et al., 1982a). DEHP (purity: analytical grade; 3,750, 7,500, 15,000 mg/kg) or MEHP (purity: > 99%; 375, 750, 1,500 mg/kg) were administered orally to pregnant animals on day 11 of

gestation, followed by the cultivation of embryonic cells for 15-20 days. Exposure to DEHP led to an aberration rate of 8, 10 and 24% and exposure to MEHP of 14, 14 and 22% in the three dose groups respectively. In the control group, 3% of the cells were aberrant. Morphological transformation showed a tendency to increase at a dose level of 3,750 mg/kg DEHP and was significantly increased at the two highest dose levels. For MEHP, the transformation ratio was significantly increased at 375 and 750 mg/kg.

In a dominant lethal test, male CD-1 mice (10 per group) were given DEHP or MEHP as a single dose of 12,500 and 25,000 mg DEHP/kg (purity: not stated) or 175 and 350 mg MEHP/kg (purity: not stated) by gavage (Hamano et al., 1979). On the day after administration and over the following 6 weeks, the males were mated with untreated females. On the 12^{th} or 13^{th} day of pregnancy, females were killed and the number of corpora lutea, of implants, of living foetuses, and of dead foetuses was determined. Pre- and postimplantation loss and dominant lethality was calculated. No significant differences between the untreated control group and treated groups were observed.

In a dominant lethal study comparable to a guideline study and performed according to GLP principles, male ICR/SIM mice (25 per group) were given 2,465, 4,930 or 9,860 mg DEHP/kg (purity: 99.7%), 50, 100 or 200 mg MEHP/kg (purity: 94.7%), or 250, 500 or 1,000 mg 2-EH/kg (purity: 99.7%) by gavage in corn oil for 5 consecutive days (Nuodex, 1981b). The control group received the corn oil vehicle daily. A positive control group was also included. After the treatment period, 20 males from each group were mated with 2 untreated females for one week and this procedure was repeated every week for a total of 8 weeks. The females were sacrificed 14-17 days after the first day of mating and scored for pregnancy, living foetuses, early and late deaths and for intercurrent infections. The mutagenic effect was determined by total implants (live foetuses plus early and late foetal deaths), total death (early and late foetal deaths), and number of dead implants per total implants. All the examined parameters were within the normal range and DEHP, MEHP and 2-EH did not appear to be mutagenic in the mouse following oral exposure.

Male ICR mice (10 per group) were given DEHP (purity: not stated) as a single dose of 12,530, 18,790 or 25,060 mg/kg by intraperitoneal injection (Singh et al., 1974). Each male was caged with two virgin adult females, which were replaced weekly with two new virgin females during a 12-week period. Between days 13 and 17 of gestation, the pregnant mice were sacrificed and examined for number of corpora lutea, total number of implantations, early and late foetal deaths, preimplantation losses, and viable foetuses (litter size). The mutagenic effect was determined directly from the increased number of early foetal deaths in individual mice, and indirectly by the reduced number of total implantations. The incidence of early foetal deaths was increased and the number of implantations was markedly reduced during the first three weeks in the highest dose group. Studies on chromosomal effects *in vivo* are summarised in **Table 4.37**. Abbreviations are explained below the table.

Table 4.37 Chromosomal effects *in vivo*

Species	Protocol	Results	Reference
rat, F344 5 males per group	cytogenetic assay 5 days, gavage in corn oil 0.5, 1.7, 5.0 ml/kg/day (500, 1,700, 5,000 mg/kg/d**) GLP	negative for CA	Putman et al. (1983), Nuodex (1981i)

Table 4.37 continued overleaf

Table 4.37 continued Chromosomal effects *in vivo*

Species	Protocol	Results	Reference
Syrian hamster females	cytogenetic assay single dose at 11th day of gestation; 0, 3,750, 7,500, 15,000 mg/kg	positive for CA, CT	Tomita et al. (1982a)
mouse, CD-1 10 males per group	single dose by gavage, 12,500, 25,000 mg/kg	negative for dominant lethal effect	Hamano et al. (1979)
mouse, ICR/SIM 25 males per group	5 days, gavage 2,465, 4,930, 9,860 mg/kg; comparable to guideline study, GLP	negative for dominant lethal effect	Nuodex (1981b)
mouse, ICR 10 males per group	single dose by ip 12,530, 18,790, 25,060 mg/kg	positive for dominant lethal effect	Singh et al. (1974)
Drosophila melanogaster Canton-S	SLRL, feed 18,600 ppm exposure period not given	negative, the percentage of lethals was 0.07 compared to 0.11 in the control group	Zimmering et al. (1989)*
Drosophila melanogaster Canton-S	SLRL injection, single dose 20 ppm	negative the percentage of lethals was 0.03 compared to 0.05 in the control group	Yoon et al. (1985)*

* Studies which are only included in the table;
** Quoted from IUCLID;
mg/kg/d mg/kg bw/day;
CA Chromosomal aberration;
CT Cell transformation;
ip Intraperitoneal;
SLRL Sex linked recessive lethal

4.1.2.8.3 Studies in humans

The frequency of chromosomal aberrations in blood lymphocytes were investigated in ten workers employed from 10-30 years in a DEHP production plant in Germany (Thiess and Fleig, 1978). Exposure levels ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3). In comparison to a control group of 20 workers, no evidence of an increased frequency of chromosome aberrations could be found. This study is considered inadequate for evaluation of genotoxicity to DEHP in humans because of the small number of workers examined and the low exposure levels.

4.1.2.8.4 Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

(see **Table 4.38**).

DEHP (purity not stated) was tested in *Saccharomyces cerevisiae* for gene conversion in strain D7-144 using two different cell culture conditions at concentrations from 1,541 to 12,325 nl/ml (1,510-12,080 µg/ml) with and without metabolic activation with rat S9 (Mehta and van Borstel, 1985). When cultured in YEPD medium pH 6.3, DEHP induced gene conversion with and without metabolic activation but not when cultured in buffer pH 7.0. According to the authors, overall test results were positive.

DEHP and MEHP were examined for activity in the C3H/10T½ murine fibroblast cell transformation system in concentrations from 0 to 100 µM DEHP (purity: 99.8%) (0-39 µg/ml) or 50-1,500 µM MEHP (purity: not stated) (14-418 µg/ml) (Sanchez et al., 1987). DEHP and MEHP did not produce cell transformation, initiate transformation in cultures treated with a tumour promoter or promote transformation in cultures pre-treated with a chemical carcinogen.

DEHP (purity: 99.9%) was tested for the ability of inducing *in vitro* morphological transforming potential using the Balb/c-3T3 clone A31 mouse cell line with (0.1, 0.3, 1.0 µl/ml (98-980 µg/ml)) and without (0.01, 0.1, 1.0 µl/ml (9.8-980 µg/ml)) metabolic activation with rat S9 (Nuodex, 1981f). The transformation frequency was not significantly increased in the presence of S9 whereas significantly increased and dose-related frequencies of transformed foci were observed in the absence of S9.

DEHP and MEHP were tested in cultured Syrian hamster embryo (SHE) cells at concentrations from 3 to 100 µM DEHP (purity: not stated) (1.2-39 µg/ml) or 1-300 µM MEHP (purity: not stated) (0.28-84 µg/ml) with and without metabolic activation with rat S9 (Tsutsui et al., 1993). Morphological transformation was significantly induced by treatment for 2 hours with DEHP at 30 and 100 µM and with MEHP at 200 and 300 µM in the presence of S9. In the absence of S9, DEHP produced a low level of transformation at 3 and 10 µM.

DEHP was evaluated in the Syrian hamster embryo (SHE) cell transformation assay in three different laboratories using the same basic experimental protocol with minor modifications at concentrations from 13 to 4,000 µg/ml (Jones et al., 1988). In one laboratory, DEHP induced a high level of transformation in two assays but gave only one transformed colony at a single dose (> 1,000 µg/ml) in a third assay. In a second laboratory, transformation was observed generally at concentrations above 1,000 µg/ml. In a third laboratory, a low number of transformed colonies were observed in two assays. It was concluded by the authors that DEHP was positive for induction of morphological transformation in this assay.

DEHP and MEHP (purity: not stated) induced morphological cell transformations when evaluated in the Syrian hamster embryo (SHE) cell transformation assay at concentrations from 0 to 75 µM (DEHP: 0-29 µg/ml; MEHP: 0-21 µg/ml) (Sanner et al., 1991).

Table 4.38 Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

Species	Protocol	Results	Reference
Fungi			
S. cerevisiae PV-2, PV-3, PV-4a,b	1-1,000 µg/ml -/+ S9 (rat)	negative for gene conversion and mitotic recombination (PV-2, PV-3); illegitimate mating induction (PV-4a, PV-4b)	Inge-Vechtomov et al. (1985)*
S. cerevisiae D7	0, 40, 200, 1,000, 5,000 µg/ml -/+ S9	negative, mitotic cross over and aberrant colonies; negative, mitotic gene conversion at 40-1,000 µg/ml; positive, mitotic gene conversion at 5,000 µg/ml	Arni (1985)*
S. cerevisiae D7	up to 50 µl /ml (49 µg/ml)	negative mitotic segregation	Parry, (1985)*
S. cerevisiae D7-144	1,541, 3,081, 6,163, 12,325 nl/ml (1,510-12,080 µg/ml); -/+ S9	negative / positive for gene conversion, see text	Mehta and van Borstel (1985)

Table 4.38 continued overleaf

Table 4.38 continued Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

Species	Protocol	Results	Reference
Fungi			
S. cerevisiae D7	200, 500, 1,000, 2,000, 3,000, 5,000 µg/ml; -/+ S9	negative, mitotic gene conversion positive, (questionable) mitotic segregation +S9	Parry and Eckardt (1985)*
S. cerevisiae RS112	mitotic recombination 0, 3000, 10,000, 30,000, 100,000, 200,000 µg/ml; -/+ S9 (rat)	negative, frequencies of deletions; interchromosomal recombinations	Carls and Schiestl (1994)*
Aspergillus nidulans diploid strain P1	mitotic recombination 0, 2,465, 4,930, 9,860 µg/ml	negative	Carere et al. (1985)*
Mammalian cells			
primary rat tracheal epithelial cells	CTA 37.5 µg/ml	Positive	Steele et al. (1989)*
mouse fibroblasts C3H/10T½	CTA 0-100 µM (39 µg/ml)	negative	Sanchez et al. (1987)
mouse fibroblasts C3H/10T½	CTA 10, 20, 40 µg/ml; -S9 250, 500, 1,000 µg/ml; +S9	negative	Lawrence and McGregor (1985)*
mouse embryo cells Balb/c-3T3	CTA 0, 0.9, 3.5, 7.0, 12.0, 21.0 µg/ml, - RLC; 0, 10,000, 25,000, 50,000 nl/ml (98,00-49,000 µg/ml), + RLC	negative	Matthews et al. (1985)*
mouse Balb/c-3T3 clone A31 cells	CTA 0.1, 0.3, 1.0 µl/ml (98-980 µg/ml) + S9 (rat); 0.01, 0.1, 1.0 µl/ml (9.8-980 µg/ml) - S9 GLP	negative + S9 positive - S9	Nuodex (1981f)
mouse Balb/c-3T3 clone I13 C14 cells	CTA 0.498, 4.98, 12.5, 24.9, 49.8 µg/ml; GLP	negative	Nuodex (1981c)*
mouse Balb/c-3T3 clone I13 C14 cells	CTA 1.64-32.8 µg/ml; GLP	negative	Nuodex (1981d)*
Syrian hamster embryo cells (SHE)	CTA 0, 3, 10, 30, 100 µM (1.2-39 µg/ml), -/+ S9	Positive	Tsutsui et al. (1993)
Syrian hamster embryo cells (SHE)	CTA 13-4,000 µg/ml	Positive	Jones et al. (1988)

Table 4.38 continued overleaf

Table 4.38 continued Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

Species	Protocol	Results	Reference
Mammalian cells			
Syrian hamster embryo cells (SHE)	CTA 0-75 µM (0-29 µg/ml)	Positive	Sanner et al. (1991)
Syrian hamster embryo cells (SHE)	CTA 0.01, 0.1, 1.0, 10, 100 µg/ml	Positive 0.2-0.9% transformed colonies in the three highest concentrations	Barrett and Lamb (1985)*
Syrian hamster embryo cells (SHE)	CTA 0.8-300 µg/ml	Positive transformation frequency up to 6%	Sanner and Rivedal (1985)*
Syrian hamster embryo cells (SHE)	CTA 77 µM (30 µg/ml)	Positive transformation in 12/1,197 colonies	Mikalsen and Sanner (1993)*
Chinese hamster V79 cells	inhibition of metabolic cooperation assay; 0.5-200 µg/ml	positive	Elmoore et al. (1985)*
Chinese hamster V79 cells	inhibition of metabolic cooperation assay up to 300 nM (0.12 µg/ml)	negative	Kornbrust et al. (1984)*
Chinese hamster V79 cells	inhibition of metabolic cooperation assay, 0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 mM (3.9-195 µg/ml)	negative	Umeda et al. (1985)*
rat, F344 4 males	stimulation DNA synthesis single dose, gavage 1.73 mmol/kg (676 mg/kg)	increased ratio of thymidine incorporation compared to control	Büsser and Lutz (1987)*
rat, F344 5 or 10 males per group	replicative DNA synthesis 1, 2, 4, 8, 18, 77, 151, 365 days, diet 1.2% (500 mg/kg/d**)	pulse-labelling technique: labelling index of hepato- cyte nuclei significantly increased only at 2 days pump infusion technique: significant increase in hepatic nuclear labelling at 8 days	Marsman et al. (1988)*
rat, F344 6 males per group	replicative DNA synthesis 8 weeks, diet 1.2% (600 mg/kg/d**)	urinary bladder epithelium labelling index unaffected	Hagiwara et al. (1990)*
rat, F344 males	replicative DNA synthesis single dose, gavage or sc 1,000, 2,000 mg/kg	replicative DNA synthesis in hepatocytes was increased at both doses at 24 hours but not at 39 and 48 hours after administration	Uno et al. (1994)*
rat, Alderley Park 10 males and 10 females	replicative DNA synthesis 14 days, gavage in corn oil 2,000 mg/kg/day comparable to guideline study, GLP	significant reduction in ^{3}H-thymidine uptake in hepatocytes from males and in proximale tubule cells in males and females	ICI (1982b)*

Table 4.38 continued overleaf

Table 4.38 continued Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

Species	Protocol	Results	Reference
Mammalian cells			
primary rat hepatocytes	stimulation of DNA-synthesis 200 µM (78 µg/ml)	Positive	Reddy et al. (1992)*
mouse, B6C3F1 48 males per group	replicative DNA synthesis 2, 8, 24, 40 weeks, diet 0, 6,000, 12,000 ppm (1,000, 2,000 mg/kg/d**)	hepatocyte labelling index: significantly elevated at 12,000 ppm (24,40 weeks) thymidine kinase activity in liver: at 12,000 ppm, increase at 2 weeks, decrease at 8 weeks; at 6,000 ppm, increase at 2 and 40 weeks	Ward et al. (1988)*
Marmoset 5 males, 5 females	replicative DNA synthesis 14 days, gavage in corn oil 2 ml/kg/d (1,960 mg/kg/d**) comparable to guideline study, GLP	no difference in ^{3}H-thymidine uptake between control and test animals	ICI (1982b)*

* Studies which are only included in the table;
** Quoted from IUCLID;
CTA Cell transformation assay;
S. cerevisiae *Saccharomyces cerevisiae*

4.1.2.8.5 Summary and evaluation of mutagenicity

The possible genotoxic effect of DEHP has been thoroughly investigated in several different short-term tests. The major metabolites of DEHP, MEHP and 2-EH, have also been examined. Most of the studies are performed according to GLP principles and are comparable to guideline studies.

The results have been negative in the majority of the *in vitro* and in *vivo* studies on DEHP, MEHP and 2-EH for detection of gene mutation, DNA damage, and chromosomal effects. The more conclusive positive results were obtained on cell transformation, induction of aneuploidy, and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic substances such as tumour promoters and/or peroxisome proliferators. Taken together all the results, both negative and positive, DEHP and its major metabolites are considered to be non-mutagenic substances.

The data available on genotoxicity do not suggest a classification of DEHP according to the criteria for classification and labelling of dangerous substances (Annex IV to Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC).

4.1.2.9 Carcinogenicity

Numerous studies on the carcinogenicity/mechanisms of carcinogenicity of DEHP have been performed *in vivo* and *in vitro* and are summarised in the **Table 4.39** and **Table 4.44**. The studies considered critical for the risk assessment; and performed according to the guideline of Annex V to the Council Diretive 67/548/EEC, or comparable to guideline studies and/or performed according to GLP principles; are described thoroughly in the text. When expressing results, the term “significant” is used only if the result is statistically significant at a p-level lower than 0.05.

4.1.2.9.1 Studies in animals

Long-term studies

Inhalation

The only inhalation study available (Schmezer et al., 1988, see **Table 4.39**) is on hamsters, and is considered inadequate for risk assessment as:

- only one dose of DEHP was used in the study;
- this dose was very low (continuous inhalation of 15 μg/m^3 for 23 months). According to the authors: "The aim of the study was to use dose range of environmentally relevant exposure similar to the human situation in indoor circumstances, and much higher concentrations may occur at some work places for short time periods";
- MTD was not reached as no signs of any toxicological effects were reported.

Oral

The Moore studies (rats, 1996 and mice, 1997):

Recently two long-term carcinogenicity studies in rats and mice have been conducted by Corning Hazleton Incorporated (CHV). The studies are comparable to guideline studies and performed according to GLP.

In the rat study, groups of F-344 rats (70 - 85 males and females per group) received in their diets DEHP concentrations of 0, 100, 500, 2,500 or 12,500 ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day, respectively, for males, and 0, 7.3, 36.1, 181.7, or 938.5 mg/kg/day, respectively, for females) for 104 weeks. In an additional recovery group, rats (55 males and females/group) were administered 12,500 ppm DEHP for 78 weeks, followed by a 26-week recovery period in which they were fed basal diet alone. In addition to the study of all relevant parameters for a carcinogenicity study, DEHP-induced cell proliferation and peroxisome proliferation in the livers of dosed rats was evaluated.

In the 2,500 ppm dose group, in both male and female rats, induction of peroxisome proliferation, increased absolute and/or relative liver weights, and increased absolute and relative kidney weights were reported. An increase in the incidence of hepatocellular adenomas and mononuclear cell leukemia (MCL, a known spontaneous neoplasm in F-344 rats) was also observed in the males of this group. In the high-dose group, 12,500 ppm, the following non-neoplastic changes were observed in both sexes: increased incidence of clinical abnormalities, increased absolute and relative mean liver weights, diffuse hepatocellular enlargement, induction of liver peroxisome proliferation, increased absolute and relative kidney weights, accompained by an increase in the incidence and severity of mineralisation of the renal papilla (only males), and increased severity of chronic progressive nephropathy (only males). Also absolute and relative testis weights were decreased, associated with increased incidence of bilateral aspermatogenesis, and decreased incidence of interstitial cell neoplasms. The incidence of hepatocellular adenomas and carcinomas were increased in both males and females of this group (first detected at week 79). The incidence of MCL was also increased in the male rats as compared with the concurrent controls or with the historical controls of the same Laboratory. In the recovery group (12,500 ppm), at the end of the study, peroxisome proliferation activity, diffuse hepatocellular enlargement and increased cytoplasmic eosinophilia in the livers of both males and females were comparable to the controls. The incidence of hepatocellular

adenomas/carcinomas for both male and female recovery animals was decreased as compared to the animals in the high dose group (12,500 ppm). In contrast to the liver, the treatment-related changes in the kidney, testis and pituitary were not reversible, and the incidence of mononuclear cell leukemia increased further in the recovery animals. The LOAEL for tumour induction (total male rats with hepatocellular neoplasms and MCL) and for the effects on the liver, kidney and testis in this rat study is 2,500 ppm DEHP in the diet (147 mg/kg bw/day for males). An overall NOAEL for the tumour induction and for the effects on the liver, kidney and testis was established as 500 ppm DEHP (29 mg/kg bw/day for male rats). The tumour incidence are presented in **Table 4.40**, the historical control data for MCL incidence in **Table 4.41**, and MCL incidence in rats exposed to different phthalates in **Table 4.42**.

In the mice study, groups of 70 male and female B6C3F1 mice received in their diets DEHP concentrations of 0, 100, 500, 1,500 or 6,000 ppm (0, 19.2, 98.5, 292.2, or 1,266.1 mg/kg/day, respectively, for males, and 0, 23.8, 116.8, 354.2, or 1,458.2 mg/kg/day, respectively, for females) for 104 weeks. In an additional recovery group, the mice (55 males and 55 females) were administered 6,000 ppm DEHP for 78 weeks, followed by a 26-week recovery period in which they were fed basal diet alone. In addition to the study of all relevant parameters for a mouse carcinogenicity study, DEHP-induced cell proliferation and peroxisome proliferation in the livers of dosed mice was evaluated.

At the end of the study, in the 500 ppm animals, induction of peroxisome proliferation but not hepatocellular proliferation, (both sexes), increased liver weights (males), and increased incidence of hepatocellular neoplasms (males, significant when compared with the concurrent controls) were observed. In the two high dose groups, 1,500 and 6,000 ppm, the effects on the liver including increased liver weights, induction of peroxisome proliferation but not hepatocellular proliferation, and increased incidence of hepatocellular adenomas and carcinomas were more pronounced in both sexes. In these two dose groups, decreased testis weight indices with an increased incidence and severity of bilateral hypospermia associated with an increased incidence, in the epididymis, of immature/abnormal sperm forms and hypospermia was also observed. Kidney weight indices (males of the 1,500 ppm-dose group and both sexes of the 6,000 ppm-dose group) were decreased and the incidence and/or severity of chronic progressive nephropathy were increased (both sexes of the both dose groups). In the 6,000 ppm recovery animals, at the termination of the study, liver peroxisome proliferation activity was comparable to control animals. Mean absolute and relative liver weights at study termination remained increased in this group compared to the control mean values, although the increase was less severe than that seen in 6,000 ppm group. The incidence of hepatocellular adenomas, but not carcinomas, was less in the 6,000 ppm recovery mice when compared to the animals in 6,000 ppm group. The decrease in testis weights and associated hypospermia was less severe in 6,000 ppm recovery males compared to 6,000 ppm animals. In the kidney, the severity of chronic progressive nephropathy, but not the effects on the kidney weights, was decreased in males and females of the recovery group compared to the 6,000 ppm animals. The LOAEL and the NOAEL for tumour induction (total male mice with hepatocellular neoplasms) in this study is 1,500 and 500 ppm DEHP in the diet, respectively (corresponding to 292 and 98 mg/kg bw/day for males of the two dose groups respectively). The LOAEL and the NOAEL for non-neoplastic effects on the liver in this study is 500 and 100 ppm DEHP in the diet, respectively (98 and 19 mg/kg bw/day for males of the two dose groups respectively). The tumour incidence is presented in **Table 4.40**.

The NTP studies (rats and mice, 1982):

The carcinogenicity of DEHP has been tested in rats and mice in the US National Toxicology Program (NTP, 1982; Kluwe et al., 1982 and Kluwe et al., 1983). The studies are comparable to

guideline studies and performed according to GLP. Fischer 344 rats and B6C3F1 mice (50 animals of each sex per group) were fed diets containing 6,000 or 12,000 (rats) and 3,000 or 6,000 (mice) ppm of DEHP (purity: > 99.5%) for 103 consecutive weeks. Controls (50 animals of each sex and species) were fed the diet without DEHP. The study was initiated when the animals were approximately 6 weeks old. All of the animals were given the control diet for 1-2 weeks after the 103 weeks of treatment and were then sacrificed and examined both grossly and microscopically. Histopathological analyses were conducted on all animals sacrificed or discovered dead during the study and included examination of skin, lungs, bronchi, trachea, bone, bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate, seminal vesicles or uterus, testis or ovary, brain, thymus, larynx and oesophagus.

For rats, the survival rate was unaffected by the test substance administration. Mean body weights of male rats in the two dose groups and that of the high-dose female rats were marginally to moderately lower than those of the corresponding controls throughout the latter three-fourths of the study. Food consumption was slightly reduced in treated rats of either sex compared to the control group. Mean daily ingestion of DEHP was calculated to be 322 and 674 mg/kg bw/day for low- and high-dose male rats, respectively, and to 394 and 774 mg/kg bw/day for low- and high-dose female rats, respectively. Seminiferous tubular degeneration and testicular atrophy were observed in 90% of high-dose male rats compared to 2 and 5% in the control group and low-dose group, respectively. Hypertrophy of cells in the anterior pituitary (cytoplasmic enlargement) was observed in 45% of high-dose male rats compared to 2 and 0% in the control group and low-dose group, respectively. The incidence of animals with foci of clear cell changes in the liver was increased in both sexes in a dose-related manner. No other non-neoplastic pathological lesions were observed. Treatment with DEHP caused liver tumours in both males and females. The incidences of hepatocellular carcinomas in male rats were 1/50, 1/49 and 5/49 (control-, low-, and high-dose group, respectively). Pairwise comparisons did not indicate a significant increase whereas a significant dose-related trend effect was observed. In female rats, the incidences of hepatocellular carcinomas were 0/50, 2/49 and 8/50 (control-, low-, and high-dose group, respectively). Pairwise comparisons revealed that the incidence in high-dose females was significantly greater than that in controls and there was a significant dose-related trend effect. Metastatic hepatocellular carcinomas were not observed. The incidences of neoplastic nodules in male rats were 2/50, 5/49 and 7/49 (control-, low-, and high-dose group, respectively) but the incidence was not significantly increased when evaluated by either pairwise comparisons or trend tests. The incidences of neoplastic nodules in female rats were 0/50, 4/49 and 5/50 (control-, low-, and high-dose group, respectively). The incidence was significantly greater in high-dose females than in the controls and displayed a significant dose-related trend effect. When combined, the incidence of rats with either hepatocellular carcinomas or neoplastic nodules exhibited a significant dose-related trend effect in both sexes and was significantly greater than controls for high- and low-dose female rats and for high-dose male rats by pairwise comparisons. An increase, not statistically significant, in the incidences of Myelomonocytic Leukemia was also observed in both males and females of the treated groups. The incidences of male rats with pituitary tumours, thyroid C-cell tumours, or testicular interstitial-cell tumours were all significantly lower in DEHP-treated animals as compared with the controls (pairwise comparisons or by trend tests). A NOAEL for DEHP-induced tumour development in the rat has not been identified as the lowest dose in the study resulted in an increase of the incidence of liver tumours. The LOAEL for tumour induction in rat is 6/000 ppm DEHP in the diet (320 mg/kg bw/day for male rats). The tumour incidence is presented in **Table 4.43**.

For mice, the survival rate was unaffected in male mice, but several low-dose female mice died after 75-90 weeks of treatment; however, these deaths were not attributed to DEHP administration. At the end of the study, mean body weights of female mice were marginally to moderately lower than those of the controls. Food consumption was similar in treated groups and the control group. Mean daily ingestion of DEHP was calculated to be 672 and 1,325 mg/kg bw/day for low- and high-dose male mice, and to 799 and 1,821 mg/kg bw/day for the low- and high-dose female mice. Seminiferous tubular degeneration and testicular atrophy were observed in 14% of high-dose male mice compared to 2 and 4% in the controls and low-dose group, respectively. A significantly higher incidence (20%) of chronic inflammation of the kidney was observed in high-dose male mice compared to 2 and 4% in the controls and low-dose group, respectively. Non-neoplastic lesions were not observed in female mice. Treatment with DEHP caused liver tumours in both sexes. The incidences of hepatocellular carcinomas in male mice were 9/50, 14/48 and 19/50 (control-, low-, and high-dose group, respectively). The incidence was significantly higher in high-dose males than that in controls and a significant dose-related trend was observed. The incidences of hepatocellular carcinomas in female mice were 0/50, 7/50 and 17/50 (control-, low-, and high-dose group, respectively). The incidences were significant at both dose levels and a significant and dose-related trend was observed. Pulmonary metastases were observed in seven low-dose and five high-dose male mice and in 1 low-dose and 7 high-dose female mice. The incidences of hepatocellular adenomas in male mice were 6/50, 11/48 and 10/50 (control-, low-, and high-dose group, respectively) and in female rats 1/50, 5/50 and 1/50 (control-, low-, and high-dose group, respectively). The incidences of hepatocellular adenomas did not differ from controls in both sexes. When combined, the incidence of mice with either hepatocellular carcinomas or adenomas exhibited a significant dose-related trend effect in both sexes and was significantly greater than controls for both sexes at either dose groups by pairwise comparisons. Lymphomas, hemangiomas, mammary-gland adenocarcinomas, and alveolar or bronchiolar carcinomas or adenomas were also diagnosed in one or more mice, but their incidences in treated animals did not differ significantly from that in controls. A NOAEL for DEHP-induced tumour development in mice could not be identified as the lowest dose in the study resulted in a significant increase of the incidence of liver tumours. The LOAEL for tumour induction in mice is 3,000 ppm DEHP in the diet (670 mg/kg bw/day for male mice). The tumour incidence is presented in **Table 4.43**.

In conclusion, the results of four different peroral long-term carcinogenicity studies in rats and mice indicate clearly that DEHP is a hepatocarcinogen in both males and females of the two species. In rat studies, the incidence of mononuclear cell leukemia (MCL) was also increased in both sexes, significant in males of Moore- study only. An increase in the incidence of Leydig cell tumours in male rats exposed for DEHP has also been reported in an abstract (Berger, 1995, **Table 4.39**)

Dermal

No studies have been reported.

Table 4.39 Summary of the carcinogenicity studies with DEHP

Species	Protocol	Results	References
Rat, F344, 70 - 85 rats/sex/group	104 weeks, diet 0, 100, 500, 2,500, and 12,500 ppm (0, 6/7, 29/36, 147/182, 789/939 mg/kg bw/day for males/females)	↑ incidence of hepatocellular adenoma and carcinomas ↑ incidence of mononeuclear cell leukaemia	Moore (1996) for more details see Table 4.40 and/or the text
Mouse, B6C3F1 70mice/sex/group	104 weeks, diet; 0, 100, 500, 1,500, 6,000 ppm (0, 6/7, 29/36, 147/182, 789/939 mg/kg bw/day for males/females)	↑ incidence of hepatocellular adenoma and carcinomas	Moore (1997) for more details see Table 4.40 and/or the text
Rat, F344 50 rats/sex/group	103 weeks, diet 0, 6,000, 12,000 ppm (0, 322/394, 674/774 males/females; mg/kg bw/day)	↑ incidence of hepatocellular carcinomas and neoplastic nodules ↓ incidence of pituitary, thyroid C-cell, and testicular interstitial-cell tumours in males	NTP (1982a), for more details se Table 4.43 and/or the text
Mouse, B6C3F1 50 mice/sex/group	103 weeks, diet 0, 3,000, 6,000 ppm (0, 672/799, 1,325/1,821 males/females; mg/kg bw/day)	↑ incidence of hepatocellular carcinomas	NTP (1982a) for more details see Table 4.43 and/or the text
Rat, Sprague-Dawley, only males	Life-time study, diet, 30, 95, 300 mg/kg bw/day	↑ incidence of Leydig cell tumours;	Berger (1995). An abstract with few experimental details.
Rat, F344, only females 20 animals/group	2 years, diet 0, 0.03, 0.1, 1.2% (ca. 0, 15, 50, 550 mg/kg bw/day[a])	↑ incidence of hepatocellular carcinomas and neoplastic nodules at 1.2%-dose group	Cattley et al. (1987)
Rat, F344, only males 10 treated, 8 controls	95 weeks, diet 2% (ca. 1,000 mg/kg/d[a])	liver tumours in 6/10 dosed rats and in 0/8 controls	Rao et al. (1987)
Rat, F344, only males 14 treated, 10 controls	108 weeks, diet 2% (ca. 1,000 mg/kg/d[a])	hepatocellular carcinomas and neoplastic nodules in 11/14 dosed rats and in 1/10 controls	Rao et al. (1990)
Rat, F 344, only males 4-6 rats/group Rat, Wistar, only males 4-6 rats/group	Upp to79 weeks, 2% DEHP in diet upp to79 weeks, 2% DEHP in diet	hepatocellular carcinomas in 1/4 and 2/4 given DEHP for 52 and 78 weeks, respectively. No neoplastic lesions	Tamura et al. (1990 a, b)
Syrian golden hamster, 65 treated and 80 controls/ sex	Inhalation, continuously exposure for life-time, 15 gm^3 DEHP vapour (total exposure of 7-10 mg/kg bw)	No significant differences in tumour incidence. The study is considered inadequate for risk assessment as only one dose of DEHP was used and the dose seem to be too low.	Schemezer et al. (1988)

Table 4.39 continued overleaf

Table 4.39 continued Summary of the carcinogenicity studies with DEHP

Species	Protocol	Results	References
Syrian golden hamster 25 animals/sex/group	Life-time, ip injections of 3,000 mg/kg bw every week (gr.1), every 2 weeks (gr.2), or every 4 weeks (gr.3)	No differences in the tumour incidence in the treated groups compared with the control group	Schmezer et al. (1988)

a) Quoted from IUCLID
↓/↑ Decreased/increased, respectively

Table 4.40 Tumour incidence in the Moore studies (rats, 1996; mice, 1997)[a]

Tumours	Dose Groups					
	1	2	3	4	5	6
RATS[b]	**Control**	**100 ppm**	**500 ppm**	**2,500 ppm**	**12,500 ppm**	**Recovery**
Number examined	80	50	55	65	80	55
Hepatocellular carcinoma						
Males	1 (1%)	0	1 (2%)	3 (5%)	24 (30%)	7 (13%)
Females	0	1 (2%)	0	1 (2%)	14 (18%)	4 (7%)
Hepatocellular adenoma						
Males	4 (5%)	5 (10%)	3 (6%)	8 (12%)	21 (26%)	12 (22%)
Females	0	3 (6%)	1 (2%)	2 (3%)	8 (10%)	6 (11%)
Total number of rats with						
Hepatocellular tumours						
Males	5 (6%)	5 (10%) [d]	4 (7%)	11 (17%) [d,e]	34 (43%)[d,e]	18 (33%) [d,e]
Females	0	4 (8%) [d]	1 (1%)	3 (5%)	21 (26%)[d]	9 (16%) [d]
MCL						
Males						
Females	15 (19%)	13 (26%)	16 (29%)	32 (49%)[d,e]	27 (34%)[d]	29 (53%)[d,e]
	14 (18%)	17 (34%)	11 (20%)	16 (25%)	17 (21%)	18 (33%)
MICE[c]	Control	100 ppm	500 ppm	1,500 ppm	6,000 ppm	Recovery
Number examined	70	60	65	65	70	55
Hepatocellular carcinoma						
Males	4 (6%)	5 (8%)	9 (14%)	14 (22%)	22 (31%)	12 (22%)
	3 (4%) 4 (6%)	2 (3%) 10 (17%)	3 (5%) 13 (29%)	10 (15%) 14 (22%)	16 (23%) 19 (27%)	
	0	2 (3%)	4 (6%)	9 (14%)	34 (49%)	
	8 (11%)	14 (23%)	21 (32%)[d]	27 (42%)[d,e]	37 (53%)[d,e]	
	3 (4%)	4 (7%)	7 (11%)	19 (29%)[d]	44 (63%)[d]	
						23 (42%)
						3 (6%)
						13 (24%)

Table 4.40 continued overleaf

Table 4.40 continued Tumour incidence in the Moore studies (rats, 1996; mice, 1997)[a]

Tumours	**Dose Groups**					
	1	**2**	**3**	**4**	**5**	**6**
RATS[b]	**Control**	**100 ppm**	**500 ppm**	**2,500 ppm**	**12,500 ppm**	**Recovery**
Hepatocellular carcinoma						
Males						14 (26%)[d]
						30 (55%)[d]
Females						
Hepatocellular adenoma						
Males						
Females						
Total number of mice with						
Hepatocellular tumours						
Males						
Females						

a) The studies (104 weeks, via diet) are comparable to guideline studies and performed according to GLP. The table was modified from text table 6, Moore (1996) and text table 7, Moore (1997).

b) Doses in mg/kg bw /day for RATS
group 1,Control
group 2, males: 5.8 mg/kg bw/day; females: 7.3 mg/kg bw/day
group 3, males: 28.9 mg/kg bw/day; females: 36.1 mg/kg bw/day
group 4,males: 146.6 mg/kg bw/day; females: 181.7 mg/kg bw/day
group 5, males: 789.0 mg/kg bw/day; females: 938.5 mg/kg bw/day
group 6, recovery: As group 5 for the first 78 weeks, followed by the basal diet alone for 26 week.

c) Doses in mg/kg bw/day for MICE
group 1, Control
group 2, males: 19.2 mg/kg bw/day; females: 23.8 mg/kg bw/day
group 3, males: 98.5 mg/kg bw/day; females: 116.8 mg/kg bw/day
group 4,males: 292.2 mg/kg bw/day; females: 354.2 mg/kg bw/day
group 5, males: 1266.1 mg/kg bw/day; females:1458.2 mg/kg bw/day
group 6, recovery: As group 5 for the first 78 weeks, followed by the basal diet alone for 26 week.

d) statistically significant different from study control, Fisher's Exact Test, $P \leq 0.05$ (Aristech Chemical Corporation and Eastman Chemical Company, 1998)

e) statistically significant different from historical control, Fisher's Exact Test, $P \leq 0.05$ (Aristech Chemical Corporation and Eastman Chemical Company, 1998)

Table 4.41 Historical controls from Covance laboratory (the same laboratory that performed Moore studies) Mononuclear Cell Leukemia (MCL) in rats:*

Study Number	**117DE**	**215DE**	**216DE**	**219DE**	**217DE**	**175DE**	**193DE**	**281DE**
MALE RATS								
Animals/study	50	50	59	58	50	50	48	55
MCL	12	18	22	21	8	15	12	20
%	24%	36%	38%	36%	16%	30%	25%	36%
Study Number	**117DE**	**212DE**	**216DE**	**219DE**	**217DE**	**175DE**	**193DE**	**281DE**
FEMALE RATS								
Animals/study	50	49	60	60	50	50	50	55
MCL	5	16	8	21	7	10	11	14
%	10%	33%	13%	35%	14%	20%	22%	25%

* Modified from Aristech Chemical Corporation and Eastman Chemical Company (1998)

Table 4.42 Incidence of Mononuclear Cell Leukemia (MCL) in rats exposed for different phthalates

Compound	**Study design**	**dose [a] (mg/kg bw/day)**	**% animals with MCL**	**Reference**
Di (2-ethylhexyl) phthalate (DEHP)	diet, 104 weeks diet, 103 weeks	146.6 Up to 674.0	males: 49% females: N.S. N.S. [b]	Moore, 1996 NTP, 1982a
Disononyl phthalate (DINP)	diet, 103 weeks	152.0 184.0	males: 60% females: 38%	Lington et al., 1997
Diallyl phthalate (DAP)	gavage, 5 d/week, 103 weeks	100.0	males: N.S. females: 51%	NTP, 1985
Butyl Benzyl phthalate (BBP)	diet, 103 weeks diet, 103 weeks	ca 600.0 (12,000 ppm in diet) Up to 24,000 ppm i	males: [c] females 36% N.S.	NTP, 1982,b NTP, 1997

a Lowest dose with a statistically significant increased MCL incidence.
b N.S.: Not significantly different from the control group.
c Surviving males were killed at weeks 29-30 because of poor survival in the dosed groups

Table 4.43 Tumour incidence in the NTP studies (1982a)[a]

Tumours	**Control**	**Low dose**	**High dose**	**P value[d]**
RATS[b]	**0 ppm**	**6,000 ppm**	**12,000 ppm**	
Hepatocellular carcinoma				
Males	1/50 (2%)	1/49 (2%)	5/49 (10%)	< 0.05
Females	0/50 (0%)	2/49 (4%)	8/50 (16%)	< 0.005

Table 4.43 continued overleaf

Table 4.43 continued Tumour incidence in the NTP studies (1982a)[a]

Tumours	Control	Low dose	High dose	P value[d]
RATS[b]	**0 ppm**	**6,000 ppm**	**12,000 ppm**	
Neoplastic nodules				
Males	2/50 (4%) 0/50 (0%) 13/50 (26%) 10/50 (20%)	5/49 (10%) 4/49 (8%) 20/50 (40%) 14/50 (28%)	7/49 (14%) 5/50 (10%) 17/50 (34%) 17/50 (34%)	not sign. < 0.05 not sign. not sign.
Females				
Myelomonocytic Leukemia				
Males				
Females				
MICE[c]				
Hepatocellular carcinoma	**0 ppm**	**3,000 ppm**	**6,000 ppm**	
Males	9/50 (18%)	14/48 (29%) 7/50	19/50 (38%) 17/50	< 0.05
Females	0/50 (0%)	(14%)	(34%)	< 0.0001
Hepatocellular adenoma	**6/50 (12%)**	**11/48 (23%)**	**10/50 (20%)**	**not sign**
Males	1/50 (2%)	5/50 (10%)	1/50 (2%)	not sign
Females				

a) The table was modified from WHO (1992). The studies (103 weeks, via diet) are comparable to guideline studies and performed according to GLP.
b) RATS Low dose, males: 322 mg/kg bw/day; females: 394 mg/kg bw/day
High dose, males: 674 mg/kg bw/day; females: 774 mg/kg bw/day
c) MICE Low dose, males 672 mg/kg bw/day; females 799 mg/kg bw/day
High dose, males 1,325 mg/kg bw/day; females 1,821 mg/kg bw/day
d) Probability level in Cochran-Armitage test for linear trend when $P < 0.05$

Studies on tumour initiating and/or promoting activity

Since DEHP is considered to be a non-genotoxic substance, it has been suggested that the carcinogenic effect is exerted during the promotion phase of hepatocarcinogenicity. DEHP has therefore been tested in several initiation/promotion experiments in rats and mice where the end-point has been the number and/or volume of altered liver cell foci. The studies are summarised in **Table 4.44**. In conclusion, DEHP have no tumour initiating activity, a positive promoting activity in mice liver and a weak or no promoting activity in rat liver.

Table 4.44 Summary of the Studies on tumour initiating and/or promoting activity

Species	Protocol	Results	References
Rat liver			
F344 rats, 10 females/gr.	Initiating test, 10 g DEHP/kg bw at 6, 12, or 24 hours, single oral dose ; 2-acetyl-aminofluorene (AAF) was used as positive promoting chemical and di-ethylnitrosamine (DEN) as positive initiating chemical. Initiating test, 1.2% DEHP (ca. 600 mg/kg bw/day), 12 weeks exposure in the diet; Phenobarbital (PB) was used as promoting chemical and diethyl-nitrosamine (DEN) as positive initiating chemical.	No tumour initiating activity of DEHP observed. Number and volume of preneoplastic foci was incresed in the positive control group. No tumour initiating activity of DEHP observed. Number and volume of preneoplastic foci was incresed in the positive control group.	Garvey et al (1987)
F344 rats, 6-18 males/gr.	Initiating and promoting test, 24 weeks exposure, 12,000ppm DEHP (ca 550 mg/kg bw/ day) in the diet, Phenobarbital (PB) was used as positive promoting chemical and N-2-fluorenylacetamide (FFA) as positive initiating chemical.	No initiating, promoting, or sequential syncarcinogenic effect was reported	Williams et al. (1987)
Sprague-Dawley (SD) rats, 5 females/gr.	Promoting test, 10, 100, 200, or 500 mg DEHP/kg bw by gavage in olive oil, 3 times a week, for 11 weeks, di-ethylnitrosamine (DEN) was used for initiation.	A weak promoting effekt was noted (a 2-fold ↑ in the number and area of ATPase-deficient foci	Oesterle and Deml (1988)
SD rats, 8-10 females and males/gr.	Promoting test, 50, 200, 500, 1,000 or 2,000mg DEHP/kg bw by gavage in olive oil, 3 times a week, for 7-11 weeks; di-ethylnitrosamine (DEN) was used for initiation.	A weak promoting effekt was noted in some treated groups (↑in the number and area of ATPase-deficient foci and GGTase-positive foci in the lower dose groups but were ↓ in the higher dose groups).	Gerbracht et al. (1990)
F344 rats, 18-20 males/gr.	Promoting test, 3,000ppm DEHP (ca. 150 mg /kg bw) in the diet for 6 weeks, di-ethylnitrosamine (DEN) was used for initiation. All rats were partially hepatectomised to maximise any interaction between proliferation and the effect of the tested compound.	No promoting effect was reported	Ito et al. (1988)
F344 rats, 10 females/gr.	Promoting test, 1.2% DEHP (ca. 600 mg/kg bw), in diet for 3 or 6 months, di-ethylnitrosamine (DEN) was used for initiation and phenobarbital (PB) as positive promoting control	No promoting effect was reported	Popp et al. (1985)
Mouse liver			
B6C3F1 mice, 30 females and 30 males/gr.	Initiating test, single dose of 25,000 or 50,000 mg/kg bw,by gavage. Phenobarbital (PB) was used as promoting chemical Promoting test, 3,000, 6,000, or 12,000 ppm DEHP (ca. 600, 1,200, or 2,400 mg /kg bw), in diet for 2, 4, or 6 months, di-ethylnitrosoamine (DEN) was used for initiation	No initiating activity was reported. Positive promoting activity (numerous foci and neoplasms were seen in mice given DEHP after DEN	Ward et al. (1983a)

Table 4.44 continued overleaf

Table 4.44 contiued Summary of the Studies on tumour initiating and/or promoting activity

Species	Protocol	Results	References
Mouse liver			
B6C3F1 mice, 29 males/gr.	Promoting test, 3,000 ppm DEHP (ca. 1,200 mg /kg bw), in diet for 28, 84 or 168 days; di-ethylnitros-amine (DEN) was used for initiation.	Positive promoting activity after only 28 days of exposure (significant and time-dependent ↑ in incidence of focal hepato-cellular proliferative lesions; sinificant ↑ in incidence of liver tumours at 168 days.	Ward et al. (1984)
B6C3F1 mice, 2 males/gr.	Promoting test, 12,000 ppm DEHP (ca. 2,400 mg /kg bw), in diet for 6 months, di-ethylnitrosamine (DEN) was used for initiation. Phenobarbital (PB) was used as positive promoting chemical.	Positive promoting activity (liver tumours were seen in mice given DEHP after DEN) A pronounced peroxisome proliferation were observed in non-tumourous liver in DEN- and DEHP-treated mice and also in the liver tumours induced by DEHP alone but not in liver tumours after DEN initiation and DEHP promotion.	Schuller and Ward (1984)
B6C3F1 mice, 30 males/gr.	Promoting test, 6,000 ppm DEHP (ca. 1,200 mg /kg bw), in thediet for 29 weeks, di-ethylnitrosoamine (DEN) was used for initiation	Positive promoting activity (↑ in incidence of focal hepatocellular proliferative lesions(FHPL) and number of FHPL per area); 4 mice given DEN and DEHP had hepatocellular adenomas	Hagiwara et al (1986)
B6C3F1 mice, 48- 55 males and females offspring, 6 weeks of age.	promoting test, 6,000 ppm DEHP (ca. 1,200 mg /kg bw), in diet for up to 78 weeks of age, transplacental initiation: N-nitrosoethylurea (NEU), ip to pregnant C57BL/6NCr mice. DNA synthesis: 6 males from each group were given 200 mg/kg bw of 5-bromo-2'-deoxyuridine (Brdu), ip, for determination of the DNA synthesis in kidneys tubular cells and in the hepatocytes	Positive promoting activity (↑ in incidence of focal hepatocellular proliferative lesions(FHPL) including hyperplastic foci, hepato-cellular adenomas and carcinomas). Authors Concl.: tumour promotion in liver may be a consequence of ↑ DNA synthesis in initiated or focus cells rather than in non-proliferative hepatocytes.	Ward et al. (1990)
C3H/HeNCr mice, females and males	Promoting test, 12,000 ppm DEHP (ca. 2,400 mg /kg bw), in diet for 26 weeks, N-nitrosodiethylamine (NDEA) was used for initiation.	Positive promoting activity (↑ in incidence of liver tumours in mice given NDEA and DEHP compared with mice exposed to NDEA alone	Weghorst et al. (1993/1994)
Rat, kidney and bladder			
Fischer 344 rats, 20 males/ gr	Promoting test, 1.2% DEHP (ca. 600 mg/kg bw), in diet for 24 weeks, N-ethyl-N-hydroxyethylnitrosamine (EHEN) was used for initiation	Positive promoting activity (↑ in incidence of renal cell adenomas and denocarcinomas and the number of tumours per kidney, in rats given DEHP after EHEN).	Kurokawa et al. (1988)

Table 4.44 continued overleaf

Table 4.44 contiued Summary of the Studies on tumour initiating and/or promoting activity

Species	Protocol	Results	References
Rat, kidney and bladder			
F344 rats, 15 males/gr.	Promoting test (uracil-accelerated transitional cell proliferation model), 0.3, 0.6 or 1.2% DEHP (ca. 250, 300, or 600 mg /kg bw), in the diet during experimental weeks 5-8 and weeks 12-20, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) was used for initiation	No promoting activity	Hagiwara et al (1990)

↓/↑ Decreased/increased, respectively

Studies on peroxisome proliferation

It has been suggested that a mechanistical association could exist between peroxisome proliferation and the occurrence of liver tumours in rodents. Therefore, the effects of DEHP on the liver (peroxisome proliferation, hepatomegaly, and replicative DNA-synthesis) have been investigated in a large number of studies. The *in vivo* studies are summarised in **Table 4.30** and the *in vitro* studies in **Table 4.31** (see also Sections 4.1.2.6.2 and 4.1.2.9.3). In conclusion, DEHP induce proxisome proliferation in the livers of experimental animals and there is species differences with respect to this effect.

4.1.2.9.2 Studies in humans

In a mortality study (prospective cohort), eight deaths were observed (expected values of 15.9 and 17.0 from the city and county data, respectively) among 221 workers exposed to DEHP for periods of 3 months to 24 years (average 11.5 years) (Thiess et al., 1978c). One carcinoma of the pancreas and one bladder papilloma were reported. No information about exposure levels is given in the report. In two other reports by Thiess et al. (1978a,b) examining the DEHP exposed workers (see Section 4.1.2.6.2), exposure levels ranging from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3) are given. The mortality study is considered inadequate for evaluation of carcinogenicity to DEHP in humans due to small cohort size, short follow-up, and low exposure levels.

Recently, occupational exposure to polyvinyl chloride (PVC) and other products in the plastics industry were assessed in a case-control study on testicular cancer using self-administered questionnaires (148 cases and 315 controls) (Hardell et al, 1997). An increased risk was observed for exposure to PVC (an increased odds ratio of 6.6; 95% confidence interval, 1.4-32), but not for other types of plastics. A possible association between exposure to DEHP and other phthalates (used as plasticisers in PVC), a potential oestrogenic effect of these chemicals, and the increased risk of testicular cancer was discussed by the authors.

In conclusion, the human data is inadequite for risk assessment.

4.1.2.9.3 Proposed mechanisms of hepatocarcinogenicity

The mechanisms through which peroxisome proliferators (PPs) such as DEHP induce liver tumours in rodents have been extensively studied and discussed in the last years A brief overview of the current opinion on mechanisms and the significance for humans will be outlined

here, for further details are referred to relevant reviews and criteria documents (Doull et al, 1999; Cattley et al., 1998; Youssef and Badr, 1998; Wolfgang et al., 1996; IARC, 1995; ATSDR, 1993; Bentley et al., 1993; ECETOC, 1992; WHO, 1992).

Negative results have been obtained in the majority of the genotoxicity studies on DEHP, MEHP and 2-EH. More conclusive positive results were obtained on cell transformation, induction of aneuploidy, and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic substances such as tumour promoters and peroxisome proliferators.

Taken together all the results, both negative and positive, DEHP and its major metabolites are considered to be non-genotoxic substances (see Section 4.1.2.8). The results of tumour initiating and/or promoting studies indicate that DEHP have no tumour initiating activity, positive promoting activity in mice liver and a weak or no promoting activity in rat liver.

In the past, generally, two mechanisms have been proposed to account for liver carcinogenesis induced by DEHP and other PPs in rodents: 1) induction of peroxisome proliferation leading to oxidative stress and generation of electrophilic free radicals, and 2) increased hepatocyte proliferation/suppression of hepatocellular apoptosis which could lead to fixation of a previously existing DNA-damage, enhancing the conversion rate of initiated cells to tumor cells, as well as increasing the susceptiblity of hepatocytes to replication and subsequent neoplastic transformation. Recently a third mechanism through activation of peroxisome proliferator-activated receptors (PPARα) has been accepted by most of the experts in this field. The possible mechanisms of hepatocarcinogenicity and the species differences with respect to these hepatic effects of PP will be discussed in this section.

Peroxisome proliferation

Peroxisomes are cytoplasmic organelles present in all cell types and contain a number of hydrogen peroxide generating oxidases, catalase (which catalyses the degradation of hydrogen peroxide), and a fatty acid β-oxidation enzyme system. Peroxisome proliferation is characterised by increased peroxisome volume density (resulting from an increase primarily in the number of peroxisomes, although size may also be increased), changes in morphology, and induction of peroxisomal enzyme activities. Studies on DEHP induced peroxisome proliferation are described in Section 07, and summarised in **Table 4.30** and **Table 4.31**. Other reported effects of PPs in hepatocytes of rats and mice include mitochondrial proliferation (with changes in enzyme activities), increase in the number of lysosomal bodies (with changes in enzyme activities and lipofuscin deposition), and induction of some microsomal enzyme activities. While marked effects have been observed in hepatocytes, only minor effects have been observed in certain other tissues. The term PPs covers substances, which in rodents induce peroxisome proliferation and liver enlargement (hepatomegaly), the latter being due to both hepatocyte hyperplasia (increased replicative DNA synthesis and cell division) and hypertrophy. Hepatomegaly and peroxisome proliferation is an early event during exposure to DEHP and has been observed in rats from about 14 days of exposure and throughout the exposure period at dose levels from 0.05% DEHP in the diet.

It has been suggested that liver tumour formation following prolonged administration of PPs arises from a sustained oxidative stress to rodent hepatocytes due to an imbalance in the production and degradation of hydrogen peroxide. The imbalance in the hydrogen peroxide production and degradation might be a result of that catalase is induced to a much lesser extent than peroxisomal β-oxidation enzymes. The increased level of hydrogen peroxide in hepatocytes might, either directly or via other reactive oxygen species (e.g. hydroxyl radicals) cause DNA damage and subsequent neoplastic transformation, or apoptosis, e.g. by oxidative damage to

intracellular membranes which then can lead to increased cell turnover thus increasing the probability of spontaneous tumour formation or to cell death.

However, there is evidence suggesting that the level of oxidative damage *in vivo* may be too low to account entirely for the carcinogenicity of PPs. Following prolonged administration (up to 79 weeks) of DEHP to rats (Tamura et al., 1990a,b), a 20-fold increase in peroxisomal β-oxidation activity was found after 2-4 weeks of treatment with a gradual decrease from week 20 to week 79 but remaining at an 8-10-fold higher level than control levels. Catalase activity increased (2-3-fold) after short-term treatment and remained at this level throughout the treatment period. The hepatic hydrogen peroxide level also increased but only 1.2-1.7-fold. As the hepatic hydrogen peroxide levels increased only slightly and did not correspond to the increase in peroxisomal β-oxidation activity, these results indicate that a large part of the hydrogen peroxide produced by peroxisomal β-oxidation could be rapidly scavenged by catalase. This could be explained by the fact that the maximal hepatic catalase activity *in vitro* is thousands of times greater than the corresponding β-oxidation activity in untreated animals. After treatment with peroxisome proliferator, the maximal activity of catalase is still thousands of times greater, even though catalase is only up-regulated 2- 3-fold and acyl-CoA oxidase increases as much as 20-fold. This indicates that liver tumours produced after long-term administration of DEHP might not be due only to the oxidative stress introduced by the enhanced peroxisomal β-oxidation. This conclusion is strengthened by the fact, that even when oxidative stress was induced by the administration of buthionine sulfoximine at a dose that drastically lowered the endogenous glutathione pool in liver, the potent peroxisome proliferator, nafenopin, failed to induce unscheduled DNA synthesis, or increase the level of DNA single strand breaks in hepatocytes from animals treated in vivo (Nilsson et al., 1991). These authors also suggested, that the modest increase in the levels of the oxidation product 8-hydroxydeoxy-guanosine (8-OHdG), and that was found in liver DNA from animals treated with the potent peroxisome proliferators over long periods of time (Kasai et al., 1989), most probably represents an artifact from isolation of the DNA where no precautions had been taken to prevent e.g. OH-radicals to be formed through Fenton-like reactions (Nilsson et al., 1991).

In conclusion, induction of peroxisome proliferation alone is not enough to explain the hepatocarcinogenic activity of DEHP in rodents.

Hepatocyte proliferation and apoptosis

Hepatocyte proliferation (characterised by increased replicative DNA synthesis and cell division, and hypertrophy) is an important response in rodent liver to PPs and has been implicated in the mechanims of rodent hepatocarcinogenesis. Hepatocyte proliferation occurs in the tumours that develop in rats and mice after administration of PPs and is seen in lesions that are the direct progenitors of tumours Thus, at least for the more potent PPs, a close correlation has been found between induction of sustained replicative DNA synthesis and the potency of various PP with respect to induction of liver tumors (Marsman et al., 1988, 1992). Howevere, the association of cell proliferation with tumour formation in rodent liver is complex and the magnitude of response for hepatomegaly and hepatocyte proliferation is not entirely predictive of eventual tumour yield. For PPs, it is important to differentiate short-term from prolonged stimulation of cell replication. Hepatomegaly is evident during the first few days of administration of PPs and is largely due to transient hepatocyte proliferation that subsides after several days as liver weight reaches a new plateau. Hepatomegaly has also been seen in rodent liver after prolonged administration of some, but not all, PPs. There are some indications that suppression of hepatocellular apoptosis also occurs during the induction of hepatomegaly. The increase in liver weight is dependent on continued exposure and is reversible upon cessation of exposure and it

has been suggested that this reversal could be related to large increases in hepatocellular apoptosis.

An increased rate of cell proliferation can be a critical effect both in tumour initiation, by increasing the frequency of spontaneous mutations and the rate of conversion of DNA adducts into mutations before they are repaired, and in tumour promotion by facilitating the promotion of initiated cells. This effect could be strengthened by suppression of hepatocellular apoptosis, which lead to increasing the number of mutant hepatocytes susceptible to replication and subsequent neoplastic transformation. It has been reported recently (James et al., 1998), that DEHP and its metabolite MEHP can induce DNA synthesis and inhibit hepatocyte apoptosis in rats and mice hepatocytes in both *in vivo* and *in vitro* studies. Also a tumour-promoting activity of DEHP was observed in the liver of mice whereas a promoting activity in the liver of rats is equivocal (see Section 4.1.2.8.1.2). As first suggested by Préat et al. (1986a, 1986b), in addition to cell proliferation PPs appear to have an important role in promoting the selective growth of basophilic preneoplastic foci. Thus, whereas phenobarbital causes an increase in the number of preneoplastic foci in a liver initiated by e.g. diethylnitrosamine, a potent PP like nafenopin or WY-14,643 does not increase the number of such foci appreciably, but causes a great increase in the size of these foci. Further, the basophilic foci induced by PPs seem to have a much higher likelihood to progress to hepatocellular carcinomas by boosting the selection of transformed cells (Préat et al., 1986a, 1986b; Cattley and Popp, 1989). Development of foci and adenomas depends on the continuous exposure to PPs, where cessation of exposure results in disappearance of benign lesions, and continued exposure is essential for progression to malignant tumors (Marsman and Popp, 1994; Miller and Cattley, 1996).

In conclusion, induction of hepatocyte proliferation combined with suppression of hepatocellular apoptosis could play a major role in the hepatocarcinogenicity of DEHP.

Activation of PPARα

The mechanism by which PPs induce peroxisome and hepatocyte proliferation as well as hepatocarcinogenicity is unclear. Howevere, recent investigations have demonstrated the central role of a class of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), in mediating the effects of PPs (reviewed by Cattley et al., 1998). In the presence of PPs or fatty acids, the PPAR receptors induce the transcriptional regulation of PP-responsive genes. Out of four different subtypes, the subtype PPARα, that is strongly expressed in tissues catabolising fatty acids (liver, digestive mucosa, kidney proximal tubules, muscle and retina), seems to be the most important with respect to processes associated with peroxisome proliferation. The expression of PPARα may be, to some extent, affected by glucocorticoids (Lemberger et al., 1994, 1996), providing an explanation of the previously observed effects on peroxisome proliferation induced by fasting and stress caused e.g. by hypothermia (de Duve, 1983; Reddy and Lalwani, 1983). Gene transcription is affected through a heterodimeric receptor complex involving PPAR α and the retinoid X receptor (RXR) that is activated by PPs and 9-cis-retinoic acid (present endogenously). The activated receptor complex regulates transcription via binding to the promoter regions of peroxisome responsive genes, e.g. those involved in the ß-oxidation of fatty acids. However, the fact that potent PPs will act as hypolipidemic drugs in man without causing detectable peroxisome proliferation seems to indicate, that other genes are also affected by PPARα, e.g. the promoter regions of the human apolipoprotein genes (apo A-I, apo A-II, apo C-III). The fact that long-term administration of DEHP in the feed down to a concentration of 200 ppm increases the dolichol content of lysosomal membranes (Edlund et al., 1986) also bears evidence of the multifaceted effects induced by PPs.

When DEHP (1.2% in the diet) was administered to Sv/129 mice entirely lacking the PPARα, none of the responses typical for peroxisome induction (such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly) found in the wild type mouse could be detected, (Ward et al., 1998). Further, whereas the wild type mouse fed DEHP exhibited typical lesions in liver, kidney and testis, no signs of liver toxicity was detected in the "knockout mouse". On the other hand, evidence of lesions in kidneys and testes were also found in the latter, indicating PPARα independent pathways for induction of toxicity in these organs. In another study with PPARα null mice fed the potent peroxisome proliferator, Wy-14,643 at 0.1% in the diet for 11 months, no indication of replicative DNA synthesis in the liver, and no increase in the incidence of liver tumors were observed. In contrast, after 11 months administration, 100% of the wildtype mice exhibited multiple hepatocellular neoplasms (Peters et al., 1997). Although it is established that PPARα could mediate liver cell proliferation and hepatocarcinogenesis of the studied PPs, the pathways involved have not been elucidated.

A report from 2003 (Klaunig et al, 2003) provides an in-depth analysis of the state of the science on several topics critical to evaluating the relationship between the mode of action (MOA) for PPARα agonist, such as DEHP, and the human relevance of related animal tumours. Topics include a review of existing tumor bioassay data, data from animal and human sources relating to the MOA for PPARα agonists in several different tissues, and case studies on the potential human relevance of the animal MOA data. The summary of existing bioassay data discloses substantial species differences in response to peroxisome proliferators *in vivo*, with rodents more responsive than primates. It is clear from the published studies that humans possess a functional PPARα, and that peroxisome proliferators activate the human receptor. It is clear that some of the genes modulated by these chemicals in humans differ from those regulated by rodent PPARα. The available epidemiological and clinical studies are inconclusive, but, nonetheless, do not provide evidence that peroxisome proliferators, such as DEHP, cause liver cancer in humans.

In conclusion, the available, studies on transgenic mice, indicate that PPARs may play a central role in mediating the hepatotoxic effects of DEHP, such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly. Also, it has been demonstrated that PPARs is required in mediating the hepatocarcinogenic effects of the PPs Wy-14,643 in mice.

Species differences

Marked species differences with respect to hepatic response to PPs are apparent, were rats and mice seem to exhibit the highest sensitivity. Guinea pigs and monkeys are relatively insensitive, while Syrian hamsters have demonstrated a sensitivity intermediate between these two groups of mammals. In a comparative study (14 days) of rats and hamsters, the liver weight of hamsters was significantly increased only at 1,000 mg/kg bw/day with no significant increases in peroxisomal enzyme activities, whereas a significant and dose-dependent (from 100 mg/kg bw/day) increase in rat liver weight as well as in peroxisomal enzyme activities was observed (Lake et al., 1984). In marmosets, the liver weight was not affected and microscopic examination revealed only a slight increase in peroxisomes following administration of 2,000 mg/kg bw/day for 14 days. In rats, hepatomegaly, marked peroxisome proliferation, and increased peroxisomal enzyme activities was observed following a similar dosage regimen (Rhodes et al., 1986). In another comparative study (21 days) with rats and cynomolgus monkeys, no treatment related changes in liver weight and peroxisomal enzyme activities were observed in monkeys at dose levels up to 500 mg/kg bw/day, whereas in rats marked effects on the same parameters were seen at a similar dosage regimen (Short et al., 1987). The studies of

DEHP on this aspect are described in Section 4.1.2.1.6, and summarised in **Table 4.30** and **Table 4.31**.

In vitro studies using primary hepatocyte cultures from rodents and primates have supported the in vivo findings. Thus, whereas a number of different PPs have caused peroxisome proliferation in rat and mouse hepatocytes, several investigations have demonstrated a lack of activity in primate and human cells (reviewed in: Ashby et al., 1994; IARC, 1995; Elcombe et al., 1997). MEHP, a metabolite of DEHP, did not stimulate peroxisome proliferation in human cells, although a marked response was obtained in rat hepatocytes (Butterworth et al., 1989).

The potential human response to PPs has been examined in liver biopsies obtained from patients treated with hypolipidemic drugs. Morphometric measurements in liver biopsies did not reveal evidence for peroxisome proliferation. The potential carcinogenic risk of hypolipidemic therapy with fibrates (clofibrate and gemfibrozil, both being potent PPs) has been evaluated in two limited clinical trials with no evidence for carcinogenesis obtained (IARC, 1996). No relevant data are available on humans exposed to DEHP. But in a study where liver biopsies had been obtained from dialysis patients, who are exposed to significant quantities of DEHP leached from PVC dialysis tubings, Ganning et al. (1987) reported an increased number of peroxisomes in exposed individuals. However, this claim was based on two electron micrographs from two different patients, where an apparent increase in the number of peroxisomes was found in one specimen.

In rodent liver, hepatomegaly and peroxisome proliferation require expression of functional PPARα (Lee et al, 1995). The slight or no responsiveness of human liver to some effects of PPs, such as hepatomegaly and peroxisome proliferation, could be explained by a low level of PPARα found in human livers (1-10% of the level found in rat and mouse liver), as well as observations of genetic variations that render the human PPARα receptor less active as compared to PPARα expressed in rodent liver (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt et al., 1999).

In conclusion, the available data indicate a quantitative species differences in the response to the hepatic effects of DEHP and in the activation of PPARα.

4.1.2.9.4 Summary and evaluation of carcinogenicity

No relevant studies in humans on the carcinogenicity of Di(2-ethylhexyl) phthalate (DEHP) is available.

In experimental animals, the only inhalation study available (Schmezer et al., 1988) is on hamsters, and is considered inadequate for risk assessment as only one dose of DEHP was used in the study. Also, the dose of DEHP used was very low and MTD was not reached as no signs of any toxicological effects were reported.

Following oral exposure, the carcinogenicity of DEHP has been investigated in numerous animal studies summarised in **Table 4.30**. Four long-term studies (Moore 1996, 1997; NTP, 1982a) performed in rats and mice are of good quality and are considered adequate for evaluation of carcinogenicity of DEHP in experimental animals. DEHP shows clear evidence of hepatocarcinogenicity in both sexes of rats and mice in the four different studies. The increase in tumour incidence in the liver was statistically significant and a dose-response relationship exists (see **Table 4.40** and **Table 4.43**). In rats, an increase in the incidence of mononuclear cell leukaemia (MCL) was also observed, significant in males of Moore study only (see **Table 4.40**

and **Table 4.43**). The LOAEL and the NOAEL for tumour induction in rats (both liver tumours and MCL) were established as 2,500 ppm (147 mg/kg bw/day for males) and 500 ppm (29 mg/kg bw/day for males) DEHP in the diet, respectively (Moore 1996). In mice the LOAEL and the NOAEL for induction of liver tumour is 1,500 ppm (292 mg/kg bw/day for males) and 500 ppm (98 mg/kg bw/day for males) DEHP in the diet, respectively (Moore 1997). Additionally, an increase in the incidence of Leydig cell tumours in male rats exposed for DEHP has been reported (Berger, 1995).

Based on the overall evaluation of the studies on mutagenicity (see Section 4.1.2.8) DEHP and its major metabolites can be regarded as non-genotoxic agents.

Hepatocarcinogenicity

Concerning the hepatocarcinogenicity of DEHP, previously, two different modes of action have been suggested for DEHP and other Peroxisome Proliferators (PPs):

- induction of peroxisome proliferation leading to oxidative stress and generation of electrophilic free radicals and/or
- increased hepatocyte proliferation/ suppression of hepatocellular apoptosis which could lead to fixation of a previously existing DNA-damage; enhancing the conversion rate of initiated cells to tumor cells; as well as increasing the susceptiblity of hepatocytes to replication and a subsequent neoplastic transformation.

None of these mechanistic premises provides a wholly satisfactory explanation of the mechanism of cancer induction caused by DEHP. However, in view of the available evidence, a mechanism due to oxidative stress seems to be the least likely to play a major role (Cattley et al., 1998; Youssef and Badr, 1998; IARC, 1995).

Recently a third and a more feasible mechanistic basis for hepatocarcinogenicity through activation of Peroxisome Proliferator activated Receptor alpha (PPARα) has been accepted by most of the experts in this field. Activation of PPARα is also required for the induction of peroxisome proliferation, cell proliferation, and most probably also of several other aspects of the multifaceted effects brought about by the PPs (Peters et al., 1997, Ward et al., 1998, Cattley et al., 1998). The role of PPARα in the toxicity of DEHP has been investigated, recently, in a subchronic study in PPARα-null and wild-type male Sv/129 mice (Ward et al., 1998). Whereas the wild-type mouse fed DEHP exhibited typical lesions in the liver (such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly), kidney, and testes, no signs of liver toxicity was detected in the PPARα-null mice. On the other hand, evidence of lesions in kidneys and testes were found also in the PPARα-null mice, indicating a PPARα independent pathway for induction of toxicity in these organs. It has been demonstrated in another study with knockout mice fed the potent peroxisome proliferator Wy-14,643 (Peters et al., 1997) that PPARα is required for the hepatocarcinogenicity of this substance. However, there is still no clear evidence showing that the carcinogenicity of DEHP in rodent is mediated through activation of PPARα.

Species differences are evident regarding the response to the different effects of the PPs on the liver. Rats and mice are very sensitive, Syrian hamsters appear to exhibit an intermediate response, whereas guinea pigs and monkeys appear to be relatively insensitive. The potential human response to PPs has been examined in liver biopsies obtained from patients treated with hypolipidemic drugs with no evidence of peroxisome proliferation. The low sensitivity of human liver to the effects of PPs could be explained by the low level of PPARα found in human liver

(1-10% of the level found in rat and mouse liver) and genetic variations that render the human PPARα less active as compared to PPARα expressed in rodent liver (Palmer et al., 1998; Tugwood et. al, 1996; Woodyatt et al., 1999). The potential carcinogenic risk of hypolipidemic therapy with fibrates, potent PPs, has been evaluated in two limited clinical trials with no evidence for carcinogenesis obtained. No relevant data are available on humans exposed to DEHP.

It has been suggested that the hepatocarcinogenic effects of PPs, such as DEHP, in experimental animals are rodent-specific and irrelevant for human. This position is held by a number of experts and is a defensible conclusion based on the available mechanistic data. However, the following arguments still indicate that a certain human cancer risk cannot, with certainity, be excluded:

1. The arguments for rodent-specificity of the liver tumours and the irrelevance of the experimental data for humans are based on the overall evidence available for all the PPs together. The weight of evidence available for each of the PPs, for example DEHP, is weaker.

2. The available data indicate a quantitative but not a qualitative, species variation in the expression of PPARα. Humans express PPARα in liver, albeit in levels lower than those found in rodents (Tugwood et al., 1996; Palmer et al., 1998). Therefor, a certain human cancer risk may still exist for some of the highly potent peroxisome proliferators. Also inter-individual differences in expression of human PPARα have been demonstrated (Tugwood et al., 1996). This evidence supports the conclusion reported by Vanden Heuvel (1999) "Therefore, although PPs may pose little risk to the population as a whole, the potential human carcinogenicity of these chemicals cannot be summarily ignored."

3. DEHP has shown positive activity in several cell transformation assays and this effect is correlated with inhibition of gap junctional intercellular communication. It may be argued that these effects on cell transformation and intercellular communication by DEHP points at a different mechanism of carcinogenicity independent of PPARα (Dybing and Sanner, 1997; Mikalsen and Sanner, 1993; Tsutsui et al., 1993).

4. An association between non-peroxisomal effects of PPs and the carcinogenic process could exist. Possible changes in non-peroxisomal parameters (such as mitochondrial effects; regulation of cytochrome P-452, hormonal disturbances; and effects on cellular biology and ion homeostasis) in experimental animals and the relevance of such effects to humans have not been well studied (Youssef and Badr, 1998; Eagon et al., 1996).

Most recently, a Working Group of the "International Agency for Research on Cancer" (IARC) have concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α) is not relevant to humans. Therefor, and based on the overall evaluation of the available data, the DEHP-induced liver tumours in rats and mice will not be considered in the present Risk Assessment Report on DEHP.

Leydig cell (LC) tumours in rats

An increase in the incidence of testicular interstitial cell tumours (LC tumours) was observed in rats exposed to DEHP in a long-term study (Berger 1995). In this study, 2,170 male Sprague-Dawley rats were exposed, lifelong, for the three non-genotoxic liver carcinogens DEHP, phenobarbital-sodium (PHB), and carbon tetrachloride (CCl_4) to assess their hepatocarcinogenic effects alone or in combination. DEHP at dose levels of 30, 95 and

300 mg/kg, in the diet, did not cause liver tumours but induce LC tumours. However, the study was published as an abstract and only few experimental data were given.

In another study on the antiandrogenic effects of Di(n-butyl) phthalate (DBP) in Sprague-Dawley rats (Mylchreest et al., 1999), pregnant rats were exposed for 0, 100, 250 or 500 mg/kg bw/day, by gavage, during the gestation days 12 to 21 (the prenatal period of male reproductive tract differentiation in the rat). In the 3-month-old F_1 males of the two high dose groups, LC hyperplasia and adenomas were observed (LC hyperplasia: 1 and 5 animals in the middle dose group and high dose group, respectively; LC adenomas: 0 and 2 animals in the middle dose group and high dose group, respectively) in addition to other testicular lesions. No incidences of hyperplasia or adenomas were observed in the control or in the low dose groups. The effects could have been more significant if the animals were allowed to live longer than 3 months according to the authors (Mylchreest et al., 1999) "Because Leydig cell adenomas generally have a late onset (1- to 2-year-old rats), it is possible that the occurrence of DBP-induced adenomas and hyperplasia would have been higher had the animals been allowed to age. In particular, the focal hyperplasia seen at 3 months of age may have eventually led to Leydig cell adenomas." In conclusion**,** this finding is important as the exposure to DBP is very short (only 10-days in utero) and the onset of lesions is early (the F_1 male rats were only 3 months old when they developed the tumours).

In a recently conducted two-generation reproduction toxicity range-finding study in Wistar rats (Schilling et al., 1999), DEHP (approximately 0, 110, 339 and 1,060 mg/kg bw/day) was administered, in the diet, to groups of 10 males and 10 females sexually immature animals (F_0 parental generation). Males and females from the same dose group were mated 70 days after the start of treatment. The females were allowed to litter and rear their pups (F_1 generation pups) until day 21 post partum. All male and female F_1 generation pups with the exception of one male and one female pup/litter (each first surviving pup/sex) were sacrificed on day 21 post partum. The selected pups (F_1 generation pups) were reared for at least 10 weeks to become the F_1 generation parental animals. The male animals of the F_1 generation parental animals were killed after the mating period. In the 3-month-old F_1 males, a slight (grade 2) diffuse LC hyperplasia were observed in all six high dose animals. The authors considered the lesion to be treatment-related. This DEHP-related effect is critical as the prenatal exposure period was relatively short, the onset of the lesions is early and the development of LC hyperplasia to LC tumours is possible if the 3-month-old animals had been allowed to age. This report support the results of Berger (1995) showing induction of LC tumours in Sprague-Dawley rats exposed for DEHP.

The relevance for humans of rodent LC tumours has recently been evaluated in an international workshop (summarised in Clegg et al, 1997) as well as in a published review (Cook et al, 1999). It was concluded that the pathways for regulation of the Hypothalamo-Pituitary-Testis (HPT)-axis in rats and humans are similar and hence, compounds that induce LCTs in rats by disruption of the HPT-axis pose a risk to human health with exception of two classes of compounds GnRH and dopamine agonists. Since it has been demonstrated that DEHP and other phthalates has a direct effect on the foetal testes the two latter mechanisms are not relevant for phthalates, and the induction of LC tumours in rats exposed for pthalates should be regarded as relevant to humans taking into consideration the species differences in sensitivity (Jones et al., 1993; Mylchreest et al., 1999; Foster, 1999).

In conclusion, the presented evidence for the phthalates-induced LC tumours in rats, and the possible endocrine effects of phthalates (Mylchreest and Foster, 1998; Schilling et al., 1999). Together with the fact that developing rats are more sensitive to the phthalates-induced testicular toxicity than sexually mature animals (Mylchreest et al., 1999) should be considered seriously.

Especially, when related to the limited human data of Hardell et al. (1997) suggesting an increased risk for testicular cancer in workers in PVC-industry.

MCL

The observed increase in the incidence of MCL in F344 rats is within the range of NTP's hisorical control data. However, the concurrent study control groups remains most appropriate for comparisons, and the historical control data, if considered, must be from the test laboratory itself. Therefore, the increase in the incidence of MCL in male rats (Moore 1996) may be DEHP-related, as the incidence were significantly increased compared to the study control and in addition to the historical control data from the same laboratory (see **Table 4.40** and **Table 4.41**). Additionally, it should be noted that increases in the incidence of MCL in F344 rats exposed to other phthalates, for example, diisononyl phthalate, diallyl phthalate, and butylbenzyl phthalate have been reported (see **Table 4.42**).

Whereas Ward and Reynolds (1983b) consider MCL in F344 rats as having similar pathology to an uncommon human tumour (large granular lymphocytic leukemia) and representing a unique model for study of natural tumour immunity, other experts regard MCL as F344 rats-specific, with little relevance for humans (Caldwell 1999). Based on the available data the relevance for humans of the DEHP-induced MCL in F344 rats is not clear.

Conclusions

The results clearly show that DEHP is carcinogenic in rats and mice (a statistically significant increase in the incidence of liver tumour with a dose-response relationship in rats and mice of both sexes, and an increase in the incidence of LC tumours and MCL in male rats). However, there is a plausible mechanism for the PPs-induced hepatocarcinogenicity in rodents (activation of PPARα) and there is evidence showing that humans are less sensitive to the hepatotoxic effects of PPs by the suggested mechanism. Therefor, the relevance for humans of the liver tumours in rodents induced by DEHP, a weak PPs, is regarded to be neglgible. Also the relevance of the DEHP-induced MCL in F344 rats is questionable. On the other hand, the induction of LC tumours in rats exposed for DEHP should be regarded as relevant to humans and, therefor, a careful evaluation of the orignal data of Berger (1995) is necessary before concluding the possible carcinogenic risk of DEHP.

Based on the overall evaluation of the available data, no classification for carcinogenicity is proposed.

4.1.2.10 Toxicity for reproduction

4.1.2.10.1 Male reproductive toxicity

Effects on fertility

Inhalation

In a 4-week inhalation study conducted according to OECD guideline 412 and the principles of GLP, male Wistar rats (10 rats per group) were exposed 5 days/week, 6 hours/day to 0, 0.01, 0.05 or 1 mg DEHP/litre (0, 10, 50 or 1,000 mg DEHP/m^3) (99.7% pure) as liquid aerosol (Klimisch et al., 1992) (see also Section 4.1.2.6.1.1). The males were mated to untreated females.

No effects on male fertility were observed 2 and 6 weeks after the end of exposure and no testicular toxicity was detected histologically. This study is, however, not considered adequate (see Section 4.1.2.6.2).

Oral

Rats

Wolfe et al. (2003) studied the multigenerational reproductive toxicity of DEHP in Sprague-Dawley rats. The unaudited draft was evaluated, without having access to all individual data. The methodology used in this study to a large extent complied with OECD Guideline 416. The number of animals in each test and control group was 17 males and 17 females only (the Guideline recommends a sufficient number of animals in each test and control group to yield preferably not less than 20 pregnant females at or near parturition). However, it is considered that enough pregnancies were produced in the study to assure a meaningful evaluation. Therefore the failure to achieve the desired number of pregnant animals does not invalidate the study. The F0 animals were administered the test article during a 6-week pre-mating period (according to the Guideline a dosing continued for at least 10 weeks before the mating period is required). This, however, is not considered to be a serious deviation since the study was conducted on three generations instead of two, and males of two generations (F1 and F2 animals) were dosed during complete spermatogenic cycle. Thus the study has provided satisfactory information concerning effects on spermatogenesis. The 10,000 ppm animals only completed the F1 generation and were terminated due to the inability to produce any F2 generation animals. This, however, is not considered to be a serious deviation since the number and choice of original dose levels (10,000 ppm group not included) is considered to be satisfactory for the purpose of the study. In excess of Guideline requirements, crossover cohabitation was performed on the control and selected F1/F2 animals. This is considered to be scientifically advantageous as it may provide additional data on effects on fertility. Except for females during the lactation period body weight and food consumption of parent animals was measured at limited time points approximately every second week (the Guideline recommends weekly measurements at a minimum). However, the limited number of measurements made in this study is considered to be satisfactory for the purpose of the study. At the time of termination brain and spleen were not weighed in adults or in pups. Nor where thymus weighed in pups. However, the deviation from the Guideline do not affect the scientific validity of this study. A complete necropsy was performed on all surviving control animals and 10 treated animals from each dose group for each sex (according to the Guideline full histopathology should be performed for all high dose and control animals selected for mating, and organs demonstrating treatment-related changes should also be examined in the low-and mid-dose groups). However, tissue samples from a number of 10 animals/sex/group are in this study considered enough to assure a meaningful histopathological examination. The deviations and/or omissions from the Guideline do not affect the scientific validity of this study. The study is performed according to GLP (although statement not yet signed), and considered acceptable.

DEHP (purity 99.8%) was administered in the diet at concentrations of 1.5 (Control 1 and 2), 10, 30, 100, 300, 1,000, 7,500 and 10,000 ppm to groups of 17 male and 17 female Sprague-Dawley Crl:CD®BR rats (source: Charles River Laboratories, Portage, Michigan). The control dose level was set at 1.5 ppm as this was the amount of DEHP found in the control feed. The 10,000 ppm group and their corresponding control group (Control 2) were added to the study structure after the initiation of the original seven dose groups and followed the same study design. Mating pairs were allowed to produce three litters (a, b, c) each. Animals in the F_0 generation began exposure as adults (5 weeks of age) and were bred to produce the F_1 generation ($F_{1a, 1b, 1c}$), the F_1 adults

(selected from F_{1c} weanlings) were bred to produce the F_2 generation ($F_{2a, 2b, 2c}$), and the F_2 adults (selected from F_{2c} weanlings) were bred to produce the F_3 generation ($F_{3a, 3b, 3c}$). The animals were administered the test article during the pre-mating period (6 or 10 weeks), and also during the mating-, gestation- and lactation periods for breeding of the F_1, F_2 and F_3 litters/pups until the day of necropsy (approximately 2 weeks after the last weaning). The F_1, F_2 and F_3 animals received diets containing DEHP after weaning (day 21 post partum) with the same concentration of DEHP as their parents received until necropsy. Additional non-mating males (up to three per litter) were selected from the F_{1c}, and F_{2c} litters, and were maintained following similar procedures as those for mating males, except they were not cohabited with females. The 10,000 ppm animals only completed the F_1 generation and were terminated due to the inability to produce any F_2 generation animals. A one-week cross over cohabitation was performed on the control and 10,000 ppm F_1 animals (up to 17 animals/sex/group), and on the control and 7,500 ppm F_2 animals (up to 17 animals/sex/group) in order to determine the affected sex. F_1 and F_2 animals were then paired with naive animals and received control feed during the cohabitation. Upon separation, the F_1 and F_2 animals received dosed feed.

Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, reproductive performance, anogenital distance, pup survival, sexual development, oestrous cyclicity, sperm endpoints, gross pathology, organ weights, and limited/selected histopathology. Based on measured feed consumption, mg/kg daily doses were calculated to be 0.12, 0.78, 2.4, 7.9, 23, 77, 592 and 775 mg/kg bw/day in the F_0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391 and 543 mg/kg bw/day in the F_1 animals; and 0.1, 0.47, 1.4, 4.8, 14, 46 and 359 mg/kg bw/day in the F_2 animals.

Parental data (general condition and behaviour, bodyweight, food intake)

The incidence of intercurrent deaths amongst treated F0 animals (17 animals/sex/group) was 1, 1, 0, 0, 1, 1, 0 in the 10, 30, 100, 300, 1,000, 7,500 and 10,000 ppm groups. The respective numbers for the F1 generation (17 animals/sex/group) were 0, 1, 1, 1, 1, 2, 0 in the 10, 30, 100, 300, 1,000, 7,500 and 10,000 ppm groups. The incidence of intercurrent deaths amongst treated F2 animals (17 animals/sex/group) was 1, 2, 0, 0, 1, 1 in the 10, 30, 100, 300, 1,000 and 7,500 groups. The incidence of intercurrent deaths amongst animals in the control groups was 0-2. Clinical signs were generally comparable among all groups in all generations and were not treatment-related in incidence or severity (stated by the author) Comment: No data were available to confirm this statement.

Statistically significant reductions in terminal body weights were noted in adult animals at 10,000 (F0 males: 6%; F1 mating males: 16%; F1 non-mating males: 21%; F1 females: 19%) (There were no F2 animals at 10,000 ppm), and at 7,500 ppm (F1 non-mating males: 10%; F2 mating males: 14%; F2 non-mating males: 14%; F2 females: 8-18% during Week 1-6). Statistically significant reductions in dam body weights were also noted at delivery (9-11%) and during lactation (11-20%) in F0 females at 10,000 ppm.

Parental feed consumption was generally comparable in all groups in all generations on a g/animal/day basis, but was statistically significant increased at 7,500 and 10,000 ppm on a g/kg bw/day basis, except during lactation where dam feed consumption was statistically significant decreased in F0 animals at 7,500 (17%) and 10,000 ppm (11%, PND 4-7).

Reproductive toxicity (for necropsy results see below).

F1-, F2-and F3-Mating Trial

Pregnancy indices were decreased at 7,500 and 10,000 ppm (see **Table 4.45**). None of the F1 mating pairs produced offspring at 10,000 ppm (this finding was correlated with no sperm or spermatids noted in these animals), and at 7,500 ppm statistically significant decreases in the pregnancy indices were noted for the F2 mating pairs. The total number of males per litter was decreased at 10,000 ppm in the F1a litter (26%) and at 7,500 ppm across all F1 litters combined (F1a+F1b+F1c) (approximately 20%). The total number of F1a pups per litter was decreased at 7,500 ppm (22%) and at 10,000 ppm (21%). The total number of pups per litter across all F1 (F1a+F1b+F1c) litters combined (18%) was also decreased at 7,500 ppm. There was also an increase in the number of cumulative days to deliver the F1a litter for F0 animals at 10,000 ppm.

Table 4.45 Pregnancy index F0-, F1- and F2 mating pairs (percent pregnant)

				Dose groups (ppm)				
Litter	**1.5 (Control 1)**	**10**	**30**	**100**	**300**	**1,000**	**7,500**	**10,000**
F1a	16/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	16/17 (94)	17/17 (100)	15/17 (88) vs 16/17 (94) in Control 2
F1b	17/17 (100)	16/17 (94)	16/17 (94)	17/17 (100)	17/17 (100)	16/17 (94)	16/17 (94)	12/17 (71) vs 15/17 (18) in Control 2
F1c	14/17 (82)	12/17 (71)	15/17 (88)	14/17 (82)	17/17 (100)	14/16 (88)	13/17 (76)	8/17 (47) vs 10/17 (59) in Control 2
F2a	15/17 (88)	17/17 (100)	16/17 (94)	17/17 (100)	16/17 (94)	16/17 (94)	12/17 (71)	*0/17 (0) vs 16/17 (94) in Control 2
F2b	13/17 (76)	13/17 (76)	15/17 (88)	15/17 (88)	13/16 (81)	14/17 (82)	10/17 (59)	*0/17 (0) vs 15/15 (100) in Control 2
F2c	10/17 (59)	11/17 (65)	10/17 (59)	10/17 (59)	9/16 (56)	11/17 (65)	10/17 (59)	*0/17 (0) vs 12/15 (80) in Control 2
F3a	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	*9/17 (53)	-
F3b	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	17/17 (100)	16/17 (94)	*8/17 (47)	-
F3c	11/17(65)	*16/17 (94)	13/17 (76)	12/17 (71)	8/17 (47)	12/17 (71)	6/17 (35)	-

* Statistically significant ($P < 0.05$)

At 10,000 ppm, male and female pup weights, unadjusted and/or adjusted for litter size, were decreased in the F1a, F1b and F1c litters (7-12%). At 7,500 ppm male and female pup weights, unadjusted and adjusted for litter size, were decreased in the F2c litter (14%) and combined F2a, 2b, 2c litters (10%).

Male anogenital distance (AGD) was decreased at 10,000 ppm in the F1a, F1b, and F1c pups (8-15%) and at 7,500 ppm in the F1a and F1b pups (6.6-8%), in the F2a and F2c pups (13-17%) and in the F3a pups (13%). No changes were noted in the female AGD throughout all the mating trials. Retained nipples were observed in the F3c male pups (11%) at 7,500 ppm. Testes descent, vaginal opening, and preputial separation were delayed at 10,000 ppm in the F1c pups, and at 7,500 ppm in the F1c, F2c and F3c pups.

The relative length of time spent in estrous stages was statistically significant increased for the F0 females at 10, 300, 1,000 and 7,500 ppm. However, no changes were revealed in the number of females with regular cycles, cycle length, number of cycles and in number of cycling females across the dose groups as compared to the control.

F1- and F2 Crossover-Mating Trial

At 7,500 and 10,000 ppm, when treated males were crossed with nulliparous naive females, there were decreased numbers of implantation sites (54% at 7,500 ppm, 98% at 10,000 ppm), and decreased indices of mating, pregnancy (8/17 versus 15/17 at 7,500 ppm; 0/17 versus 11/17 at 10,000 ppm), and fertility (8/14 versus 15/17 at 7,500 ppm; 0/17 versus 11/17 at 10,000 ppm). At 7,500 and 10,000 ppm, when treated females were crossed with naive males, there was a decrease in AGD in the male pups (11.5% at 7,500 ppm; 17% at 10,000 ppm). Also at 7,500 ppm, male, female, and combined pup weights were decreased, both when unadjusted and adjusted for litter size (8-16%).

Sperm end-points

At terminal necropsies, various sperm end-points were found to be decreased at 7,500 ppm in the F1, F2, and F3 males and at 10,000 ppm in the F0 and F1 males. Epididymal sperm density was decreased at 7,500 ppm in the F2 (64%) and F3 males (94%), and at 10,000 ppm in the F1 males (99.6%). Comment: As a result of technical difficulties the epididymal sperm data for the 10,000 ppm F0 males was not obtained. Total spermatid/cauda was decreased at 7,500 ppm in the F1 (61%), F2 (73%) and F3 males (95%), and at 10,000 ppm in the F1 males (99.8%). Total spermatid/testis was decreased at 7500 ppm in the F1 (69%), F2 (74%) and F3 males (79%), and at 10,000 ppm in the F0 males (31%). At 10,000 ppm no spermatids were present in the testes of F1 males. Spermatid/mg testes was decreased at 7,500 ppm in the F1 (56%), F2 (57%) and F3 males (67%). Decrease in the motile percentage was noted in the F2 males (25%) at 7,500 ppm. Decrease of 12.8% in track speed was revealed along with a 15.6% decrease in the lateral amplitude in the F0 males at 10,000 ppm. Abnormal sperm morphology was seen in the F2 males at 100, 300, 1,000 and 7,500 ppm (stated by the author) Comment: No further data were available. Organ weight changes (statistically significant) noted for adult F0-, F1- and F2-animals are highlighted below (see **Table 4.46** and **Table 4.47**)

Table 4.46 F0-, F1-, F2 adult male necropsy results (% change relative to Control)

Males													
Parameter	**1,000 ppm**					**7,500 ppm**					**10,000 ppm**		
	mating			**non-mating**		**mating**			**non-mating**		**mating**		**non-mating**
	F0	**F1**	**F2**	**F1**	**F2**	**F0**	**F1**	**F2**	**F1**	**F2**	**F0**	**F1**	**F1**
Terminal body weights								↓14	↓10	↓14	↓6	↓16	↓21
Organ weights													
Absolute liver		↑13				↑43	↑25		↑21	↑14	↑47		↑14
Relative liver		↑8		↑4	↑8	↑45	↑33	↑36	↑35	↑34	↑55		↑43
Absolute kidney											↑11		
Relative kidney				↑4		↑14		↑17	↑13	↑17	↑17		↑18
Relative adrenal											↑25	↑27	↑31

Table 4.46 continued overleaf

Table 4.46 continued F0-, F1-, F2 adult male necropsy results (% change relative to Control)

Males													
Parameter	**1,000 ppm**					**7,500 ppm**					**10,000 ppm**		
	mating			**non-mating**		**mating**			**non-mating**		**mating**		**non-mating**
	F0	**F1**	**F2**	**F1**	**F2**	**F0**	**F1**	**F2**	**F1**	**F2**	**F0**	**F1**	**F1**
Absolute right cauda epididymis							↓37	↓43	↓19	↓32	↓19	↓62	↓44
Relative right cauda epididymis							↓32			↓20		↓54	↓34
Absolute right epididymis							↓35	↓36	↓20	↓27	↓16	↓55	↓54
Relative right epididymis							↓30					↓47	↓42
Absolute right testis							↓51	↓60	↓34	↓49	↓23	↓80	↓80
Relative right testis							↓47	↓53	↓28	↓40		↓76	↓75
Absolute ventral lateral prostate							↓28						↓32
Absolute dorso-lateral prostate												↓29	
Absolute seminal vesicles								↓24				↓29	
Absolute pituitary													↑19
Relative pituitary													↑47

Increased absolute liver weights were also noted in the 10 ppm F0 males (13%)

Table 4.47 F0-, F1-, F2 adult female necropsy results (% change relative to Control)

	Females										
Parameter	**300 ppm**			**1,000 ppm**			**7,500 ppm**			**10,000 ppm**	
	F0	**F1**	**F2**	**F0**	**F1**	**F2**	**F0**	**F1**	**F2**	**F0**	**F1**
Terminal body weights									↓18	↓11-20	↓19
Organ weights											
Absolute liver	↑10						↑27	↑29	↑32	↑36	
Relative liver				↑10		↑12	↑36	↑39	↑51	↑53	↑38
Absolute kidney											↓18
Relative kidney							↑12			↑15	
Relative adrenal											
Relative uterus											↑36
Relative ovaries											↑35

The results above show statistically significant organ weight changes noted in adult animals in the liver, kidney, male accessory sex organs at the 7,500 and 10,000 ppm doses, and in the liver at 10 (males only), 300 (females only) and 1,000 ppm. At 10,000 ppm organ weight changes were also observed in adrenal glands (males only), pituitary (males only), uterus and ovaries.

Statistically significant organ weight changes were also noted in F3 animals. A dose-related increase in the absolute and relative liver weights were noted in the 1,000 ppm (21% and 17%, respectively) and 7,500 ppm (51% and 63%, respectively) males. The relative liver weight was also increased (36%) in the 7500 ppm females. Absolute and relative right testis weights were decreased in the 7500 ppm males (48% and 45% respectively). Decreases in absolute dorso-lateral prostate weight (41%) and relative epididymis weights (35%) were also noted for the 7500 ppm males.

The Gross pathological examination findings of reproductive and other organs performed on the F0-mating animals (see **Table 4.48**), F1 mating animals (see **Table 4.49**), F1 non-mating males (see **Table 4.50**), F2 mating animals (see **Table 4.51**) and F2 non-mating males (see **Table 4.52**) are highlighted below Comment: no individual animal data on gross observations were available.

Table 4.48 F0 mating animals: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	10,000
Number of Animals Observed	**16 or 17**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Testis* (right) small	0	0	0	0	0	0	0	2
Testis* (left) small	0	0	0	0	0	0	0	1
Prostate (ventral) small	0	0	0	0	0	0	1	0

* It is not specified whether the findings on testis were bilateral or not. No individual animal data were available

Table 4.49 F1 mating animals: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	10,000
Number of Animals Observed	**17**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Testis (bilateral) small	0	0	0	0	0	0	7	10
Testis* (right) soft	0	0	0	0	0	0	1	0
Testis* (left) soft	0	0	0	0	0	0	1	0
Epididymis** (right) small	0	0	0	0	0	0	1	0
Epididymis** (left) small	0	0	0	0	0	0	1	0
Cauda epididymis small	0	0	0	0	0	0	1	0
Seminal vesicles small	0	0	0	0	0	0	1	0
Prostate (ventral) discoloured	0	0	1	0	0	0	0	0
Prostate (dorsolateral) small	0	0	0	0	0	0	1	0

* It is not specified whether the findings on testis were bilateral or not. No individual animal data were available
** It is not specified whether the findings on epididymis were bilateral or not. No individual animal data were available

Table 4.50 F1 non-mating males: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	10,000
Number of Animals Observed	**27 or 39**	**36**	**39**	**40**	**45**	**43**	**30**	**21**
Testis (bilateral) small	0	0	0	0	2	0	9	21
Testis (unilateral) small	0	0	0	0	0	0	1	0

Table 4.50 continued overleaf

Table 4.50 continued F1 non-mating males: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	10,000
Number of Animals Observed	**27 or 39**	**36**	**39**	**40**	**45**	**43**	**30**	**21**
Testis (right) aplasia	0	0	0	0	1	0	0	0
Epididymis (bilateral) small	0	0	0	0	1	0	0	21
Epididymis (right) aplasia	0	0	0	0	1	0	0	0
Cauda epididymis (right) small	0	0	0	0	0	0	0	21
Cauda epididymis (right) aplasia	0	0	0	0	1	0	0	0
Seminal vesicles (right) lobe missing	0	0	0	0	0	0	0	1
Seminal vesicles (right) lobe hypoplasia	0	0	0	0	1	0	0	0
Seminal vesicles (right) lobe small	0	1	0	0	0	0	0	0
Seminal vesicles (left) lobe small	0	0	0	0	1	0	0	0
Prostate (dorsolateral) small	0	0	0	0	0	1	0	1
Prostate (ventral) small	0	0	0	0	0	3	1	1

Table 4.51 F2 mating animals: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	1,0000
Number of Animals Observed	**17**	**10**	**10**	**10**	**10**	**10**	**10**	**-**
Testis (bilateral) small	0	0	0	0	0	0	8	-
Testis (unilateral) small	0	0	0	0	0	0	1	-
Epididymis (bilateral) small	0	0	0	0	0	0	8	-
Epididymis (unilateral) small	0	0	0	0	0	0	1	-
Cauda epididymis small	0	0	0	0	0	0	8	-

Table 4.52 F2 non-mating males: Number of Gross Observations

DEHP Dose (ppm)	1.5	10	30	100	300	1,000	7,500	10,000
Number of Animals Observed	**20**	**25**	**25**	**21**	**21**	**25**	**20**	**-**
Testis (bilateral) small	0	0	0	0	1	1	11	-
Testis (unilateral) small	0	0	0	0	0	2	0	-
Testis (left) soft	0	0	0	0	0	1	0	-
Epididymis (bilateral) small	0	0	0	0	1	1	7	-
Epididymis (unilateral) small	0	0	0	0	0	2	0	-
Cauda epididymis (right) small	0	0	0	0	1	1	6	-

All other gross findings seen at necropsy were considered not dose related and incidental.

Aplastic testes, epididymis and seminal vesicles, and small testes and epididymis were noted in 1-3 non-mating males at 300 ppm. At 1,000 ppm small prostates were noted in 3 or 4 non-mating males. In comparison of the incidence of these findings to TherImmune´s (the laboratory in question) historical control data, the incidence of the findings in the seminal vesicle and prostate is similar while the incidence for male testis and epididymis is increased (stated by the author).

Comment: No data were available to confirm this statement (historical control data were not included in the draft).

Histopathology

Treatment-related microscopic findings stated by the author are highlighted below. Comment: No data were available to confirm these statements (the Pathology Report including microscopic analysis was not included in the draft).

In the testes, minimal to marked atrophy of the seminiferous tubules characterized by loss of germ cells and the presence of Sertoli cell-only tubules, as well as occasional failure of sperm release, were noted at 10,000 ppm in the F0 and F1 males, and at 7,500 ppm in the F1 and F2 males. Minimal atrophy of seminiferous tubules was also observed in F1 males at 100 ppm (1/10) and at 300 ppm (1/10). The changes noted in the testes were correlated with "small testis" observed grossly in the most severe cases of F0 males, and were found in all 7,500 and 10,000 ppm F1 males. In F2 males atrophy of the seminiferous tubules, presents in 10/10 males at 7500 ppm, was correlated to the gross observation of atrophy, and there was failure of sperm release in 1/10 males. Comment: No data were reported for the 1,000 ppm group. Secondary changes were present in the corresponding epididymis including sloughed epithelial cells/ residual bodies (3/10 F0 males at 10,000 ppm; 6/10 F1 males at 7500 ppm) and aspermia (1/10 F0 males at 10,000 ppm; 4/10 F1 males at 7,500 ppm; 9/10 F1 males at 10,000 ppm). Secondary changes (including aspermia, oligospermia, residual bodies/sloughed epithelial cells) were also present in the corresponding epididymis of F2 males at 7,500 ppm (number of animals not specified).

Minimal to mild hepatocellular hypertrophy was noted at 10,000 ppm in the F0- (males: 9/10; females: 10/10) and F1 animals (males: 6/10; females 9/10), at 7,500 ppm in the F0- (males: 10/10; females 9/10), F1- (males: 10/10; females: 10/10) and F2 animals (males: 10/10; females: 10/10), and at 1,000 ppm in the F1- (males: 5/10) and F2 animals (number of animals not specified).

Dilatation of the tubules and mineralization occasionally associated with chronic pyelonephritis was observed at 10,000 ppm in the F1 animals (males: 5/10; females: 3/10), at 7,500 ppm in the F1- (males: 3/10; females: 5/10) and F2 animals (males: 4/10; females. 5/10) and at 1,000 ppm in the F1 animals (females 1/10)

Cortex vacuolisation of the adrenals was noted at 10,000 ppm in the F0- (males 6/10 versus 1/10 in the controls) and F1 animals (males 5/10 versus 1/10 in the controls), and at 7,500 ppm in the F1 animals (males 4/10 versus 2/10 in the controls). Comment: Findings in the adrenal glands (not specified) were also noted in the F1 animals at 1,000 ppm (stated by the author). No further data were available.

Results of the Pathology Working Group's (PWG's) reexamination

Sertoli cell vacuolation was observed in the control group as well as in the 1,000 ppm and 7,500 ppm F1 males. It was not observed in the 10,000 ppm animals with diffuse seminiferous tubule atrophy. In the 7,500 ppm males, Sertoli cell vacuolation was observed in seminiferous tubules without atrophy. This vacuolation was similar to that observed in the control group males Comment: The vacuolation of Sertoli cells observed resulted from disortion during fixation and processing of the tissues according to the PWG. This distortion could have obscured any minimal toxic effects that may be present.

Conclusion

The no-observed adverse effect level (NOAEL) for testicular toxicity in this study was 100 ppm (equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and approximately 5 mg DEHP/kg bw/day in the F1 and F2 animals) and was based on decreased absolute and/or relative testis weights noted at 7,500 (F1, F2 and F3 males) and 10,000 ppm (F0 and F1 males), macroscopic pathological findings (small or aplastic testes) noted at 300 (3/45 non-mating F1 males, 1/21 non-mating F2 males), 1,000 (3/25 non-mating F2 males), 7,500 (7/10 mating F1 males, 10/30 non-mating F1 males, 9/10 mating F2 males, 11/20 non-mating F2 males) and 10,000 ppm (2 or 3 of 10 F0 males, 10/10 mating F1 males, 21/21 non-mating F1 males), and microscopic pathological findings (testis seminiferous tubular atrophy) noted at 300 (1/10 F1 males), 7,500 (all F1 and F2 males) and 10,000 ppm (all F1 males, 2 or 3 of 10 F0 males).

Microscopic and/or macroscopic pathological findings and organ weight changes (absolute and/or relative) were also noted in the epididymis, seminal vesicles and prostate. Thus, macroscopically small and/or aplastic epididymis were noted at 300 (2/45 non-mating F1 males, 1/21 non-mating F2 male), 1,000 (3/25 non-mating F2 males), 7,500 (1 or 2 of 10 mating F1 males, 9/10 mating F2 males, 7/20 non-mating F2 males) and 10,000 ppm (21/21 non-mating F1 males). Small seminal vesicles were noted at 300 (1/45 non-mating F1 males) and 7500 ppm (1/10 mating F1 males), and small prostate was noted at 1,000 (3 or 4 of 43 F1 non-mating males), 7,500 (1/10 F0 mating males, 1/10 F1 mating males, 1/30 non-mating F1 males) and 10,000 ppm (1 or 2 of 21 non-mating F1 males). Microscopic pathological changes in the epididymis including sloughed epithelial cells/residual bodies and aspermia/oligospermia were found in F0 and F1 males at 7,500 and 10,000 ppm. Organ weight changes were noted in the epididymis (F1 and F2 males at 7,500 ppm; F0 and F1 males at 10,000 ppm), seminal vesicles (F2 males at 7,500 ppm; F1 males at 10,000 ppm) and prostate (F1 males at 7,500 and 10,000 ppm). At 7,500 ppm changes in epididymis and prostate weights were also noted in F3 males.

The low observed adverse effect level (LOAEL) for testicular toxicity was set at 300 ppm (equivalent to approximately 23 mg DEHP/kg bw/day in the F0 animals and 14 mg DEHP/kg bw/day in the F1 and F2 animals). At this dose level macroscopic pathological findings in testes (aplastic and/or small) were noted in animals of both generations (F1 and F2), and microscopic pathological findings in testes (seminiferous tubular atrophy) were noted in 1/10 F1 males. Further on, macroscopic pathological findings in male accessory sex organs other than testes (mentioned above) were also present at this dose level and at higher doses. Atrophy of seminiferous tubules in testis was also observed at 100 ppm. However, this effect on testis at 100 ppm was only noted in one animal in one generation (F1) and in the absence of any accompanying findings. At 300 ppm additional parameters and several generations of animals were affected. Effects on male accessory sex organs other than testis could also be taken into consideration at this dose level. Therefore the LOAEL was set at 300 ppm.

The NOAEL for fertility toxicity in this study was 1,000 ppm (equivalent to approximately 77 mg DEHP/kg bw/day in the F0 animals, and 48 and 46 mg DEHP/kg bw/day in the F1 and F2 animals respectively) and was based on impaired fertility and litter parameters noted at 7,500 ppm and above, and decreased various sperm end-points noted at 7500 (F1-, F2-, F3 males) and 10,000 ppm (F0-, F1 males). None of the F1 mating pairs produced offspring at 10,000 ppm (this finding was correlated with no spermatids present in the testes of F1 males at 10,000 ppm). At 7,500 ppm statistically significant decreases in the pregnancy indices were noted for the F2 mating pairs (8/17 vs. 17/17). The total number of males per litter was decreased at 10,000 ppm in the F1a litter (26%) and at 7,500 ppm across all F1 litters combined (F1a+F1b+F1c) (approximately 20%). The total number of F1a pups per litter was decreased at

7,500 ppm (22%) and at 10,000 ppm (21%). The total number of pups per litter across all F1 (F1a+F1b+F1c) litters combined (18%) was also decreased at 7,500 ppm. There was also an increase in the number of cumulative days to deliver the F1a litter for F0 animals at 10,000 ppm.

The NOAEL for developmental toxicity in this study was 100 ppm (equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and approximately 5 mg DEHP/kg bw/day in the F1 and F2 animals) and was based on the fact that the testicular effects were much more severe in the F1 and F2 generations than in F0, indicating the developmental phases as sensitive to the testicular toxicity of DEHP.

The NOAEL for effects not related to reproductive toxicity in adult animals was 300 ppm (equivalent to approximately 23 mg DEHP/kg bw/day in the F0 animals, and 14 mg DEHP/kg bw/day in the F1 and F2 animals) and was based on reductions in bodyweights noted in both sexes at 7,500 (F1, F2 animals) and 10,000 ppm (F0, F1 animals), absolute and/or relative organ weight changes noted at 1000 ppm and above (increased liver: 1,000 ppm and above; increased kidneys: 1,000 ppm and above; increased adrenals: 10,000 ppm; increased pituitary: 10,000 ppm), and microscopic pathological findings noted at 1,000 ppm and above (liver hypertrophy: 1,000 ppm and above; cortex vacuolisation of the adrenals: 7,500 ppm and above; dilation of the tubules and mineralization in the kidneys occasionally associated with chronic pyelonephritis: 1,000 ppm and above). Microscopic pathological findings in the adrenal glands were also indicated in F1 animals at 1,000 ppm (no further data).

Table 4.53 Summary of the testicular- fertility- and developmental-toxicity in the Wolfe study

Dose (ppm)	F0 (only exposure as adults)	F1	F2	F3 (shorter survival time)
100		testis min.atrophy 1/10		
300		small testes 2/45 testis aplasia 1/45 small epididymes 2/45 small seminal vesicles 1/45 seminal vesicle hypoplasia 1/45 testis min.atrofi 1/10	small testes 1/21 small epididymes 1/21	small testes 0/?
1,000*		small testes 0/43 small prostate 3 or 4/43	small testes 3/25 small epididymes 3/25	small testes 0/?

Table 4.53 continued overleaf

Table 4.53 continued Summary of the testicular- fertility- and developmental-toxicity in the Wolfe study

Dose (ppm)	F0 (only exposure as adults)	F1	F2	F3 (shorter survival time)
7,500	small prostate 1/10	small testes 10/30 (non-mating males) small testis 7/10 (mating males) small epididymes 1 or 2/10 small seminal vesicles 1/10 small prostate 1/10 (mating males) small prostate 1/30 non-mating males) testis atrophy 10/10 (mating males) testis atrophy 30/30 (non-mating males) epididymis histopat. changes 6/10 ↓testis weight (abs. 51%, rel. 47%) ↓epididymis weight (abs. 35%, rel. 30%) ↓prostate weight (abs. 28%) sperm reduction impaired litter parameters decreased anogenital distance affected sexual development	small testes 9/10 (mating males) small testes 11/20 (non-mating males) small epididymes 9/10 (mating males) small epididymes 7/20 (non-mating males) testis atrophy 10/10 epididymis histopat. changes ?/10 ↓testis weight (abs. 60%, rel, 53%) ↓epididymis weight (rel. 27%) ↓seminal vesicles weight (abs. 24%) decreased fertility sperm reduction decreased pregnancy indices reduced pup weights decreased anogenital distance affected sexual development	small testes 0/? ↓testis weight (abs. 48%, rel 45%) ↓prostate weight (abs. 41%) ↓epididymis weight (rel. 41%) sperm reduction decreased anogenital distance retained nipples affected sexual development
10,000	small testes 2 or 3/10; testis atrophy 2 or 3/10; epididymis histopat. changes 3/10; ↓testis weight (abs. 25%); ↓epididymis weight (rel. 16%); sperm reduction	small testes 21/21 (non-mating males) small testes 10/10 (mating males) small epididymes 21/21 seminal vesicles aplasia 1/21 small prostate 1 or 2/21 testis atrophy 10/10 epididymis histpat. changes 9/10 ↓testis weight (abs. 80%, rel. 76%) ↓epididymis weight ↓prostate weight (abs. 28%) ↓seminal vesicles weight no spermatids impaired litter parameters reduced pup weights decreased anogenital distance affected sexual development	no offspring	—

In conclusion, a NOAEL of 4.8 mg/kg/day is obtained for testicular toxicity and developmental (testicular) toxicity. The NOAEL for fertility is 46 mg/kg/day.

Results from a recently performed 2-generation reproduction toxicity study in Wistar rats indicate effects on reproductive performance, several organs, survival (overall, 8 of 50 adult high dose females died or were killed for humane reasons), as well as on development (Schilling et al., 2001). The study was performed according to current guidelines and in conformity with GLP. Wistar rats (25 rats/sex and generation) were exposed to dietary levels of 0, 1,000, 3,000 or 9,000 ppm DEHP (corresponding to approximately 0, 113, 340 or 1,088 mg/kg bw and day). The F0 animals were exposed as from the age of 37 days, for at least 73 days before mating, and until weaning. F1 pups were raised and mated to produce a F2 generation. Selected F2 male and female animals (10 of each sex) performed a functional observation battery, motor activity, and a water maze test at 21 days of age. Considering the known testicular toxicity of DEHP, effects on the testis will be presented separately (**Table 4.54**), followed by other findings. The most relevant findings are compiled in the tables below.

Histopathology of the testis was performed with light microscopy after Bouins fixation, paraplast embedding, and Haematoxylin and Eosin staining. Evaluation of the testis showed focal tubular atrophy to be the most frequent finding. In the F0 animals, the frequency was 0/25, 1/25, 3/25, and 6/25 in the control, low, mid, and high dose groups, respectively. The number of affected tubules/testis, as well as the presence of diffuse tubular atrophy, was increased in the high dose group.

In the F1 adult males, the frequency of focal tubular atrophy was 3/25, 7/25, 4/25, and 14/25 in the control, low, mid, and high dose groups, respectively. Although fewer animals were affected in the mid than in the low dose group, the effects in the mid dose animals were more pronounced than in the low dose animals. Thus, the number of affected tubules/testis was increased in the two highest dose groups. In addition, diffuse tubular atrophy was observed in the high dose group (3/25). Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. A reduced or absent sperm-/spermatid counts together with sperm abnormalities was observed in the high dose groups in 2 and 1 animal(s) (of 25) in the F0 and F1 adult males, respectively.

A reduced testis weight (absolute and relative) was observed in the high dose F2 pups.

Table 4.54 Effects on testis-related parameteres

Generation	End-point	control	1,000 ppm	3,000 ppm	9,000 ppm	Trend analyses (logistic regression, # # p<0.01, # # # p<0.001)
F0	Testis spermatid count (millions)	102	98	96	93	
	Epididymes sperm count (millions)	596	584	568	552	
	Abnormal sperms (%)	2.8	2.4	3.8	6.1	
	Pup production (male fertility) (% of control)	100	92	92	88*	
	Testicular focal tubulary atrophy	0/25	1/25	3/25	6/25	# #
F1 adults	Abnormal sperms (%)	2.6	2.5	3.1	3.3*	
	Testicular focal tubulary atrophy	3/25	7/25	4/25	14/25	# # #
F2 pup	Rel. weight of testes, day 21 p.p., (% of control)	0	-1	-3	-10**	

Other findings

F0

Observations on the F0 parental females were mortality (2/25), a decreased food consumption (25%), reduced body weights, body weight loss during lactation (14%), and a retarded body weight gain (25%) in the high dose group. Effects on organ weights and/or histopathology were observed among both females and males. Besides effects on the testis (see above), there was also an affect on the ovaries in the high dose group (reduced number of growing follicles and of corpora lutea, 15%* and 25%**, respectively). Effects on reproductive performance were evident in the high dose group, as illustrated by a reduced fertility index among both females and males, and an increased postimplantation loss.

F1 pups

The most important observations made in the F1 pups are summarised in **Table 4.55** below.

Table 4.55 Observations in F1 pups

Generation	End-point	Control	1,000 ppm	3,000 ppm	9,000 ppm
F1 -pups	Pup viability 0-4 days (% of control)	97	95	93*	93**
	Pup weight gain 4-21 days p.p. (% of control)	0	-4	-6	-36
	Pup body weight day 21 (% of control)	0	-3	-5	-31
	Sex ratio (% males of total, day 0)	51	47	57	53
	Anogenital index (% of control, males)	0	-2	-6 *	-8 *
	Presence of aerola/nipples (% affected pups/litter)	2	0	1	76**
	Time of vaginal opening (day p.p.)	30	31	31	33*
	Time of preputial separation (day p.p.)	44	45	46	52*
	Rel. weight of spleen, males, day 21p.p., (% of control)	0	-10*	-10*	-38**
	Rel. weight of spleen, females, day 21 p.p., (% of control)	0	-11*	-6	-38**
	Rel. weight of thymus, males, day 21 p.p., (% of control)	0	0	-8*	-11**
	Rel. weight of thymus, females, day 21 p.p., (% of control)	0	-3	-5	-13**

Observations on the F1 pups in the high dose group included reduced number of live (viability index) and total number of pups, increased number of stillborn pups, increased pup mortality, reduced body weights (31%) and body weight gains (36%) until weaning (day 21 post partum). Feminisation of male pups was indicated by a reduced anogenital distance (14%), a reduced anogenital index (8%), and an increased frequency of areolas/nipple anlagen in male pups. The timing of sexual maturation was delayed in both females (vaginal opening) and males (preputial separation). The weight of the thymus and spleen were reduced.

Some of these effects were also significant in the mid dose group (e.g., the viability index, the anogenital index, and the weights of thymus and spleen), and although not statistically significant in most cases, there appears to be a trend also including small effects in the low dose group.

F1 parental animals

The most important observations made in the F1 parental animals are summarised in **Table 4.56** below.

Table 4.56 Observations in F1 animals

Generation	End-point	Control	1,000 ppm	3,000 ppm	9,000 ppm
F1 adults	Female mortality/moribound condition (%)	0	0	0	24[b]
	Body weight (male) [a]	0	0	-3	- 14*
	Pup production (male fertility) (% of control)	92	100	92	76
	Stillborn pups (% of total)	0.9	0.6	6.1 *	5.1 *
	Relative weight of thymus, males, (% of control)	0	9	12	33**
	Relative weight of thymus, females, (% of control)	0	0	4	24**
	Ovarian follicle count; growing follicles	52	n.a.	51	37**
	Ovarian follicle count; corpora lutea	26	n.a.	27	20**

a) In the female, there was only an effect in the high dose group (–2%*)
b) In all 5 animals histological effects were observed in the thymus (atrophy in 3 and starry sky cells in 2)

In the high dose group, there was an increased mortality/sacrifices among dams (6/25) and malformed external genital organs in males (2/25). Food consumption, body weights, and body weight gains were reduced in both males and females. The effects on reproductive performance and organ weights/histopathology were almost identical to those in the F0 generation.

In the lower dose groups, there were besides the effects on the testis (see above) also an increased number of stillborn pups in the mid dose group.

F2 pups

The most important observations made in the F2 pups are summarised in **Table 4.57** below.

Table 4.57 Observations in F2 pups

Endpoint (F2 pup)	Control	1,000 ppm	3,000 ppm	9,000 ppm
Pup viability 0-4 days (% of control)	95	97	81*	83*
Pup weight gain 4-21 days p.p. (% of control)	0	-1	-7 (but significant at days 4-14)	-40*
Pup body weight day 21 (% of control)	0	0	-6 (sign. day 14)	-33 *
Sex ratio (% males of total, day 0)	54.1	51.4	49.8	46.3
Anogenital index (% of control, males)	0	-2	-8 *	-8 *
Presence of aerola/nipples (% affected pups/litter)	1.1	2.0	49**	59**
Rel. weight of testes, day 21 p.p., (% of control)	0	-1	-3	-10**
Rel. weight of spleen, males, day 21 p.p., (% of control)	0	3	0	-30**
Rel. weight of spleen, females, day 21 p.p., (% of control)	0	3	-2	-34**
Rel. weight of thymus, males, day 21 p.p., (% of control)	0	-7	-11*	-15**
Rel. weight of thymus, females, day 21 p.p., (% of control)	0	1	-4	-9

In the high dose group, the observations in the F2 pups were almost identical to those in the F1 pups, but included a reduced weight of the testis.

In the mid dose group, the effects in F2 pups seemed more severe than in the F1 pups. There were an increased number of stillborn pups, a decreased live birth index and viability index, lower body weights (6%) and body weight gains (7%), a reduced anogenital distance/index (9% and 8%, respectively), an increased presence of aerola/nipple anlagen affecting 49% of the males, and a decreased thymus weight in males. Although not statistically significant, there appears to be a trend also including small effects on the thymus and the testis (see also above) in the low dose group.

Timing of sexual maturation was not studied in the F2 generation.

In the high dose group, a functional observation battery performed on selected animals at day 21 post partum revealed reduced values for grip strength in males, and reduced values for landing foot-splay in both males and females. The body weights of these animals were reduced (27-38%), but it is not clear whether the reduced body weight could account for the functional effects. No effects were observed on the water maze test or on motor activity. No effects were observed in the lower exposure groups.

Evaluation of immunological data

The present study indicates that DEHP induces atrophy of spleen and thymus. There was a significant decrease in spleen weight at all doses in both male and female F1-pups and a significant decrease in thymus weight in the mid and high dose groups in F1 males. In the F1-females a significant reduction of the thymus was only observed at the highest dose level, however, a non-significant but clear dose dependent trend was observed also for the low and mid dose groups. In the F2-pups, spleenic weight was significantly reduced in the high dose group with 30 and 34% in males and females, respectively. The effect on the thymic weight in the F2-pups is similar to that in the F1-pups. A significant reduction in the mid and high dose groups of the F2-males, and for the F2-females a non-significant but dose-dependent reduction in the mid and high dose groups.

In the highest dose group the reduced spleen and thymus weights were observed in parallel with a significant reduction in male and female F1 and F2 pup body weights. Thus, it is possible that in the highest dose group the effect on spleen and thymus weights could be associated with the reduced body weight. However, the effect on the spleen observed in the low-dose group of both male and female F1 pups and on the thymus weight in the mid dose group of male F1 and F2 pups was not accompanied by a reduced body weight. Therefore, without further testing of the immunotoxicity of DEHP a direct immunotoxic effect of DEHP cannot be excluded. Thus, for the effect on the spleen a LOAEL of 1,000 ppm can be concluded from this study.

Evaluation of testicular data

In this study, significant and severe effects on testicular histology, sperm morphology, fertility, and sexual development of the offspring have been observed in the high dose group of both generations. Several of these effects are also clearly apparent in the mid dose group, e.g., a reduced testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring (as indicated by the presence of aerola/nipple anlagen in the males). Some of these effects are also occurring in the low dose group (e.g., focal tubular atrophy), although few tubuli are affected per testis. However, based on the clear dose-response, we conclude that there is an

adverse effect on the testis also in the low dose group (113 mg/kg and day), which thus constitutes the LOAEL of the study.

Overall evaluation

It should be observed that although there has been some focus on the testicular effects, the testis have only been studied by standard methods (Bouins fixation, paraplast embedding, and Haematoxylin and Eosin staining) and no measurements of, e.g. hormone concentrations have been conducted. Still, there were effects on numerous parameters relating to reproductive success in the low dose animals (testicular tubular atrophy, relative weight of testis in F2, pup production, pup viability, anogenital index, pup weight gain, and time of sexual maturation of males and females), effects that were not statistically significant in this dose group but at higher ones. Although some of these effects are small in the low dose group, the relevance is supported by known mechanisms of action and clear dose-responses involving the three dose groups.

Wistar rats have been used in the present study. Although it is not the first time Wistar rats have been used in studies on the toxicity of DEHP, the fact that none of the previous studies giving low LOAELs have used Wistar rats, gives some concern relating to the sensitivity of this strain as compared to, e.g. the more commonly used Sprague-Dawley rat.

The low dose in this study (113 mg/kg and day) is considered a LOAEL, and hence, no NOAEL can be deduced from this study.

In a study comparable to a guideline study, Agarwal et al. (1986a,b) administered DEHP (> 99% pure) to groups of 24 sexually mature male F344 rats (age about 15 weeks) in the diet at 0, 320, 1,250, 5,000 or 20,000 ppm (equivalent to doses of 0, 18, 69, 284 and 1,156 mg/kg bw) per day for 60 days. The males were mated with undosed females at exposure days 61 to 66. A dose-dependent reduction in total body, testis, epididymis, and prostate weights was observed at 5,000 and 20,000 ppm. The only functional reproductive consequence of exposure of male rats to DEHP was a significantly reduced mean litter size at 20,000 ppm (1,156 mg/kg bw/day). This effect was directly correlated with degenerative changes in the testes, along with decreased testicular zinc content, significant reduction in epididymal sperm density and motility, and increased occurrence of morphologically abnormal sperm. There was a trend towards decreased (not statistically significant) testosterone and increased luteinising hormone (LH) and follicle stimulating hormone (FSH) in serum at 5,000 and 20,000 ppm. The incidence of pregnancy, mean litter weight on day 1, frequency of stillbirth and neonatal death, and mean litter growth up to 7 days of age were unaffected. A NOAEL of 69 mg/kg bw/day is derived.

Dostal et al. (1988) assessed the fertility in mating trials in adult male rats after neonatal exposure to DEHP (0, 100, 200, 500 or 1,000 mg/kg bw/day) on days 6-10. There were no consistent changes in fertility, implantation rate, or in the number of live foetuses in untreated females mated with the DEHP-treated males. Twenty-four hours after the final dose, the Sertoli cell number was reduced; the numbers had returned to normal after 6 and 13 weeks of age. At 6 weeks there was a dose-related decrease in maturation of the spermatids in the tubules. At 13 and 19 weeks of age (but not at 11, 12, 16 or 23 weeks) there were decreases in testis weight and testicular spermatid numbers. Although marked changes in testicular weight occurred, no differences in fertility were observed at any age between DEHP-treated rats and controls. The authors concluded that the loss of Sertoli cells due to DEHP exposure neonatally did not affect the fertility of the rats as adults, but may have caused subtle effects on sperm production.

Results from a recently performed 2-generation range finding study in Wistar rats indicate effects on fertility and developmental toxicity (see also Section 4.1.2.10.4) (Schilling et al.,

1999). The study was performed according to current guidelines and in conformity with GLP. Wistar rats (F0 generation = 10 rats/sex) were exposed to dietary levels of 0, 1,000, 3,000 or 9,000 ppm DEHP (corresponding to approximately 0, 110, 339 or 1,060 mg/kg bw/day). F1 pups were raised and mated to produce a F2 generation which was sacrificed two days after birth. The mean relative liver weight was significantly increased in F0 parental males at 3,000 and 9,000 ppm (at the higher dose level also the absolute liver weight). No treatment related histopathological changes were, however, noted. There was a reduced total number of delivered F1 pups and the viability index was reduced on post partum day 0 and 4 at 9,000 ppm. In F1 male pups a treatment related loss of spermatocytes was found at 3,000 and 9,000 ppm (2/10 and 7/9, respectively). At the highest dose level, the presence of areolas/nipple anlagen was significantly increased and the male sexual maturation (based on preputial separation) was significantly retarded. A reduced anogenital distance was observed in F2 male pups at 9,000 ppm (not ivestigated in F1 pups).

Mortality occurred in F1 parental males (3/9) at 9,000 ppm in the premating phase, initially also reduced food consumption and reduced mean body weights were noted. At this dose level, the fertility was also reduced in the males (fertility/mating index 83%). The absolute and relative testicular weight and the absolute epididymidal weight were significantly decreased at 9,000 ppm. The prostate weight showed a dose-related decrease from 1,000 ppm. The testes and the epididymides were reduced in size in three out of six animals at 9,000 ppm. Histopathology revealed focal or diffuse atrophy of spermatogenesis of the testes and diffuse Leydig cell hyperplasia in all males, interstitial oedema in the testes in three out of six animals, and debris of an altered spermatogenesis in the epididymides in five out of five animals. Also aspermia (2/5), missing seminal vesicle (1/6), and areolas/nipple anlagen (1/6) were noted. There was a dose-related increase of stillborn pups from 3,000 ppm and a decrease of delivered F2 pups, statistically significant at 9,000 ppm.

The effects found in F1 parental males indicate that DEHP exerts a specific action on male genital organs such as the testicle and the epididymis, when males are exposed during early development. This is strengthened by the fact that female gonads were unaffected.

However, concerning testicular effects in developing male pups only one testicle per litter was studied histopathologically in F1 pups and none of the F2 pups. F1 pups were culled at day 21 and neither undescended testes nor hypospadia were investigated.. Neither is there any information on effects on Sertoli cells in F1 parental male rats in this range finding study as is seen in several other studies presented below (Gray and Butterworth, 1980; Sjöberg et al., 1985c; Sjöberg et al., 1986a,b; Gray and Gangolli, 1986; Dostal et al., 1988; Poon et al., 1997; Arcadi et al., 1998). However, according to preliminary data from the following main study presented by the Industry (ECBI/37/99 – Add.10), Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1,000 ppm.

Mice

Swiss (CD-1) mice (20 animals of each sex) were dosed with 0.30% DEHP (150 mg/kg bw/day; purity not specified) in the diet (Morrissey et al., 1988). Continuous breeding studies were used to evaluate reproductive performance over a 98-day cohabitant period. Mice were separated by sex during the first 7 days of DEHP treatment. After detection of an adverse effect of DEHP treatment, a 1-week crossover mating trial was carried out between previously treated males and control females. Reproductive ability was assessed at 10 weeks of age in a single breeding trial over a 7-day period. Necropsy for the treated males included organ weights, percentage motile sperm, sperm concentration, and percentage abnormal sperm. In DEHP treated mice, there was a

reduction in epididymal and testicular weights, sperm motility, and sperm concentration and an increased number of abnormal sperm cells. No further details are given.

In another fertility assessment by continuous breeding (study comparable to a guideline study and performed according to GLP principles), DEHP (> 99% pure) was given to CD-1 mice (20 animals of each sex per dose group and 40 control animals of each sex) at dietary levels of 0, 0.01, 0.1 or 0.3% (equivalent to 0, 20, 200 or 600 mg/kg bw/day, respectively) (Lamb et al., 1987) (see also later in this section and Section 4.1.2.10.3). Both male and female mice were exposed during a 7-day premating period and were then randomly grouped as mating pairs. The dosing continued for the 98-day cohabitation period and thereafter for 21 days. Reproductive function was evaluated by measuring the number of litters per breeding pair, the number of pups per litter, the proportion of pups born alive and the mean pup weight. Dietary levels of 0.1 and 0.3% DEHP produced dose-dependent and significant decreases in fertility and in the number and proportion of pups born alive. In males, 0.3% DEHP caused significantly reduced weights of the testes, epididymis, prostate, and seminal vesicles. All but one high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. Sperm analysis showed a significant decrease of percent motile sperm and a significantly decreased sperm concentration in cauda epididymis. Exposure to 0.3% DEHP also caused an increased incidence of abnormal sperm forms. DEHP did not significantly decrease body weight gain in the high-dose group. A crossover mating trial conducted with F_0 mice showed a decrease in fertility both for treated males and treated females. Only four litters out of twenty were born to treated males mated to control females and the proportion of pups born alive was decreased. No pups were born when dosed females were mated to control males. A NOAEL of 20 mg/kg bw/day is derived.

An oral two-generation study (comparable to a guideline study and performed according to GLP principles) in CD-1 mice was performed with 0.01, 0.025 or 0.05% DEHP (> 99% pure) in the diet (NTIS, 1988). The doses were equivalent to about 19, 48, and 95 mg/kg bw/day. The study was carried out to examine the effect of prenatally administered DEHP on the growth, development, and reproductive performance of the F1 generation. The F1 generation was mated within dose groups at sexual maturity and F2-offsprings were evaluated for viability and growth at postnatal day 4. For F1-litters, the percentage of prenatal mortality was increased at the high dose (9% versus 26,4%). During the neonatal period, the percent of viable pups was significantly decreased at 0.05% DEHP. No other effects of DEHP were observed upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testes decent or vaginal opening, or spontaneous locomotor activity) on postnatal days 14, 21 or 50. A NOAEL for parental toxicity and for F2-offspring was 95 mg/kg bw/day. A NOAEL of 48 mg/kg bw/day was concluded for F1-offspring.

Thirty mice of an inbred colony were used to study the effect of DEHP on reproductive function in mice (Jain and Joshi, 1991). Fifteen animals were orally dosed with 1,000 mg/kg bw of DEHP (purity not specified) in 0.1 ml olive oil for one week. The fertility (evaluated by the ability of the motile spermatozoa to fertilize normal cycling females) was reduced from 90 to 75%. Sperm density and sperm motility were also significantly reduced.

Other routes

The effects of subcutaneous administration of 1-10 ml of undiluted DEHP (purity not specified) to adult male ICR mice (8-10 weeks of age) on day 1, 5, and 10 followed by mating with untreated adult virgin females have been reported by Agarwal et al. (1985b). Control animals were injected with normal saline subcutaneously. A single mating at day 21 resulted in a reduction in the incidence of pregnancy from 87.5% in the control group to 62.5% in the 1 ml dose group and 37.5% in 2, 5 and 10 ml dose groups. However, the reduction in number of

viable foetuses per pregnancy was statistically significant only at the 1 and 10 ml/kg dose levels. Repeated matings with nulliparous females starting on day 2, 6, 11, 16 and 21, and continuously at weekly intervals, revealed no effect on the pregnancy rate. In another part of this study, male mice were injected with 1, 2, 5, 10, 20, 40, 60, 80 or 100 ml/kg body weight on days 1, 5 and 10. Control animals were injected with saline (100 ml/kg body weight). A marked reduction of vaginal plugs and a reduced pregnancy rate were seen from 20 ml/kg bw. No further details are given.

The same authors also report a similar study where 16 male mice from each group were cohabited with untreated females one to one for 7 consecutive days to evaluate fertility at dose levels of 2, 5, 15, 20, 60 or 80 ml/kg bw DEHP (purity not specified) (Agarwal et al., 1989). A dose-dependent reduction in pregnancy rate was observed from 2 ml/kg bw.

Gonadal effects

Testicular effects have been observed in several repeated dose toxicity studies in rats (Gray et al., 1977; NTP, 1982; ICI, 1982b; CMA, 1984b,c; Ganning et al., 1990; Eastman Kodak, 1992a; Moore, 1996; Poon et al., 1997). These studies are described in more detail, including systemic effects, in the section on Repeated dose toxicity (see Section 4.1.2.6). Studies (including NOAELs/LOAELs) used for the reproductive risk assessment procedure are compiled in **Table 4.58**.

Oral

Rats

In a 90-day study performed according to OECD Guidelines and the principles of GLP mild to moderate seminiferous tubular atrophy and Sertoli cell vacuolation were observed in the testes of young male Sprague-Dawley rats (Poon et al., 1997). Groups of 10 young males per dose level were given 0, 5, 50, 500 or 5,000 ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw) per day in the diet for 13 weeks. The rats were 105-130 g (approximately 32-37 days) at initiation of dosing and reached sexual maturity at 70 days (Charles River, 2000). The method for preparing testicular tissue included Zenker's fluid fixation, paraffin embedding and haematoxylin and eosin staining. The histopathological slides were controlled blindly. No clinical signs of toxicity were observed. Feed consumtion and body weight gain were not affected. At 5,000 ppm, rats of both sexes had significantly increased absolute and relative liver weights and relative kidney weight. (Non-reproductive effects in both sexes are presented in more detail in Section 4.1.2.6.)

In the 500 ppm dose group, a high incidence of minimal to mild Sertoli cell vacuolation was observed in 7 out of 10 rats. No other effects were noted at this dose level. At 5,000 ppm, the absolute and relative testis weights were significantly reduced. Microscopic examination revealed a mild to moderate, bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules in 9 out of 10 rats. The incidence and severity of seminiferous tubular atrophy were similar to those found in a following study on di-*n*-octyl phthalate with a positive control group fed a diet containing 5,000 ppm DEHP. The progressive increase in vacuolation of Sertoli cells plus injury and loss of germinal epithelium and spermiogenesis in a treatment-related fashion is regarded as a powerful evidence that the changes observed were not artifactual and that the conclusions were not compromised by the technology employed. A NOAEL of 50 ppm DEHP in the diet (3.7 mg/kg bw/day) is derived from the study.

The effects of DEHP, MEHP and 2-ethylhexanol (2-EH) were determined on gonocytes and Sertoli cell morphology, Sertoli cell proliferation, and expression of cell cycle markers in neonatal rats (three-day old, CD Sprague-Dawley) (Li et al, 2000). A single bolus dose of DEHP (20, 100, 200 and 500 mg/kg) was given in corn oil to five pups per group. Diethyl phthalate (DEP: 500 mg/kg) served as the non-toxic control. MEHP (393 mg/kg), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. The doses of MEHP and 2-EH were molar equivalent with 500 mg/kg DEHP. In this dose-response study, all pups were killed 24 hours after dosing. A time-course study was conducted following a single dose DEHP (200 mg/kg), where the pups were killed after 6, 9, 12, 24 or 48 hours.

Biochemical analyses was performed for serum FSH levels, Sertoli cell proliferation (as BrdU labelling; BrdU administered 3 hours before euthanasia), cell cycle regulators cyclin D1, D2, D3, $p27^{kip1}$ proteins and cyclin D2 mRNA in the testes. Morphological examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 hours post-treatment with DEHP (100-500 mg/kg). With 200 mg/kg DEHP these effects were first determined 12 h after treatment, and persisted for 48 hours. Effects on Sertoli cell morphology were not detailed in the report. MEHP (single dose group) induced effects on gonocytes similar to DEHP. BrdU-labelled Sertoli cells were dose-dependently decreased from 100-500 mg/kg DEHP. No marked difference in BrdU-labelled Sertoli cells was marked with 20 mg/kg DEHP, DEP and vehicle controls. Serum levels of FSH were not affected by DEHP treatment (200 and 500 mg/kg). MEHP also caused a significant decrease in BrdU-labelled Sertoli cells. D2 mRNA was specifically down-regulated by DEHP in a dose-dependent manner (200 and 500 mg/kg only doses reported), and this decrease was manifest as a small, transient but reproducible reduction in the amount of cyclin D2 protein with 200 mg/kg DEHP (only dose reported). The effects of MEHP and 2-EH were not determined. 2-EH was without effect on testicular cell morphology, or Sertoli cell proliferation.

A NOAEL for young pups of 20 mg/kg is derived for effects on altered gonocyte morphology and decreased Sertoli cell proliferation by a single oral dose of DEHP.

Akingbemi at al. (2001) studied effects of oral exposure to DEHP on male steroidogenesis in Long-Evans rats using several different exposure regimes. Hormone levels (testosterone and LH) were determined *in vivo* in serum, and Leydig cells were isolated and cultured for analyses of *in vitro* androgen biosynthesis. Pregnant dams (n=7) were administered 100 mg/kg and day of DEHP by gavage during gestation day 12 to 21. Serum levels of testosterone and LH were significantly reduced in the offspring at 21 and 35 days of age (approximately to 70 and 40% of controls levels, respectively), but not at 90 days, as measured in 9-18 randomly selected male pups per group. In Leydig cells isolated from 18 pups, the testosterone production was reduced at day 21 (by approximately 50%), but not later. After exposure of lactating dams to 100 mg/kg and day (n = 7) during postnatal day

(PND) 1 to 21 by gavage, serum concentrations of testosterone in the offspring were reduced at day 21, but not at day 35 and 90 post-exposure. No effects were seen on LH.

Prepubertal rats (n=10) were gavaged with 0, 1, 10, 100 or 200 mg/kg and day for 14 days during either PND 21-34 or 35-49. No effects were observed on serum hormone levels, but the Leydig cells were affected by DEHP as indicated by decreased *in vitro* testosterone production and inhibited steroidogenic enzymes (measured in isolated Leydig cells) after exposure to 100 or 200 mg/kg and day (exposure PDN 21-34), or 10, 100 or 200 mg/kg and day (exposure PND 35-48).

When prepubertal rats were exposed as above, but for 28 days (PND 21-48), increased concentrations of serum testosterone, interstitial fluid testosterone, and serum LH were observed (30-40 % at 10, 100 and 200 mg/kg and day). Similarly, the testosterone production was dose-dependently increased in isolated Leydig cells obtained from these rats. When young adult rats were exposed as above for 28 days (PND 62-89), no effects were observed on any parameter.

This study shows that the younger the rats are, the more sensitive they are to the effects of DEHP. Exposure of the dams during pregnancy or at the first postnatal weeks to 100 mg/kg and day reduced the serum levels of testosterone in male offspring. Effects on the Leydig cells were indicated at even lower exposure (10 mg/kg and day), but the relevance of the in vitro assay is not clear. The LOAEL for effects of DEHP on the serum concentration of testosterone in very young rats is 100 mg/kg and day.

In a study reported by Gray et al. (1977), groups of 15 male and female Sprague-Dawley rats were exposed to DEHP via incorporation in the diet at concentrations of 0, 0.2, 1.0 or 2.0% (0, 143, 737 or 1,440 mg/kg bw/day in males) from 1 up to 365 days (see also Section 4.1.2.6.1). The absolute testicular weight in mid- and high-dose rats was lower than compared to control rats while the relative weights were increased. Histological examination revealed a severe seminiferous tubular atrophy and cessation of spermatogenesis related to the dietary level of DEHP. These changes were demonstrated from week 2. A LOAEL of 143 mg/kg/day DEHP is derived from this study.

Parmar et al. (1986) also found that DEHP affects spermatogenesis in adult male albino rats. Groups of 6 adult male rats were administered 0, 250, 500, 1,000 or 2,000 mg DEHP/kg bw (purity not specified) in groundnut oil by gavage for 15 days. Body weight, testicular weight, sperm concentration, and activity of several testicular enzymes were determined. In the 2,000 mg/kg bw group, both absolute and relative weights of the testes were significantly reduced. In all dosed groups, sperm counts were significantly reduced in a dose dependent manner from 250 mg/kg bw. The activities of γ-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) were significantly increased at doses from 500 mg/kg bw, sorbitol dehydrogenase (SDH) was decreased from 1,000 mg/kg bw, and acid phosphatase was reduced at 2,000 mg/kg bw. The activity of β-glucuronidase was significantly increased at 2,000 mg/kg bw. The authors suggested that DEHP can affect spermatogenesis in adult rats by altering the activities of these enzymes responsible for the maturation of sperms and that the reduced number of sperms may be responsible for the antifertilic effects of DEHP. The authors also concluded that even at 250 mg/kg bw DEHP causes a decrease in testicular function after short-term dosing. A LOAEL of 250 mg/kg/day DEHP is derived from this study.

Siddiqui and Srivastava (1992) gavaged groups of six male rats with 0, 500 or 1,000 mg/kg bw of DEHP in groundnut oil for 15 days. The following day dosed animals were sacrificed and the epididymes were removed, weighed and processed for further study of sperm count and biochemical parameters. In the high dose group, both absolute and relative epididymis weights were significantly decreased. Sperm count showed a significant dose-related decrease in both dose groups. In the high dose group, the activity of aldose reductase and sorbitol dehydrogenase was significantly decreased and the activity of lactate dehydrogenase was significantly increased. A LOAEL of 500 mg/kg/day DEHP is derived from this study.

In a more recent oncogenicity study, performed according to EPA guidelines and in conformity with the principles of GLP, F-344 rats (70 males and females/group; approx. 6 weeks old at initiation of dosing) were administered DEHP (99% pure) at dietary concentrations of 0, 100, 500, 2,500 or 12,500 ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day) for at least 104 weeks

(Moore, 1996) (see also Section 4.1.2.7.1 and 4.1.2.9.1). An additional group (55/sex) was administered 12,500 ppm DEHP for 78 weeks, followed by a recovery period of 26 weeks.

An increased incidence of clinical abnormalities was observed in males from the two highest dose groups and the recovery group, significant at the highest dose level. There also was a decreased survival and decreased body weight gain in both sexes at these dose levels, significant only at the highest dose level. Signs of general toxicity are described in more detail in Section 4.1.2.67.1 and neoplastic effects in Section 4.1.2.9.1.

In males that died or were sacrificed in extremis during the study, there was an increased (not statistically significant) incidence of small and/or soft testis, small epididymis and/or seminal vesicle. At study termination a dose-related increase of small and/or soft testis was observed in the 2,500 and 12,500 ppm group. Also the recovery group had an increased incidence of small or soft testis. An increased incidence (not significant) of aspermatogenesis was present at 2,500 ppm in unscheduled deaths, at interim sacrifice, and at study termination. At 12,500 ppm, the absolute and relative testis weights were significantly decreased with associated increased incidence of bilateral aspermatogenesis in all males accompanied by hypospermia in the epididymis and decreased incidence of interstitial cell neoplasms (3/10 compared to 9/10 in control group). In the pituitary, an increased number of castration cells were observed in 30/60 males compared to 1/60 of the control males. There was no indication in rats killed at study termination that DEHP-related changes in the testes and pituitary were reversible upon cessation of DEHP-exposure. Due to the dose-related serious effects on the testicles, a NOAEL of 500 ppm corresponding to 28.9 mg/kg/day can be derived for testicular effects.

Fischer 344 rats (10 animals/sex/group) were given 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm (0, 80, 160, 320, 630 or 1,250 mg/kg/day) of DEHP (> 99.5% pure) in the diet for 13 weeks prior to an oncogenicity study (NTP, 1982). The mean body weight gain of male rats was depressed (29%) in males at 25,000 ppm relative to controls. Testicular atrophy was observed in all males fed 25,000 ppm and was present, but less pronounced in males fed 12,500 ppm (630 mg/kg/day). No other compound-related histopathological findings were observed. A NOAEL of 320 mg/kg/day DEHP is derived from this study.

In the following oncogenicity study, Fischer 344 rats (50 animals/sex/group; initial body weight just above 200 mg for males and around 150 mg for females) were given 0, 6,000 or 12,000 ppm (0, 322 or 674 mg/kg/day for males) DEHP (> 99.5% pure) in the diet for 103 weeks (NTP, 1982) (see also Section 4.1.2.9.1). Mean daily doses of DEHP were 322 and 674 mg/kg body weight per day for low- and high-dose male rats, respectively. The survival rate was unaffected. At the end of the study, mean body weights of dosed male rats and high-dose female rats were marginally to moderately lower than those of the corresponding controls. Food consumption was slightly reduced in rats of either sex. Interstitial-cell tumours of the testis were observed in a statistically significant negative relation to dose. There was a statistically significant increase in bilateral tubular degeneration of the seminiferous tubules and atrophy in the testes. The incidences were 1/49 (2.0%) in the control, 2/44 (5%) in the low-dose, and 43/48 (90%) in the high-dose group. Histologically, the seminiferous tubules were devoid of germinal epithelium and spermatocytes (tissues had been preserved in 10% buffered formalin and embedded in paraffin). Only Sertoli cells were seen on tubular basement membranes. Interstitial cells were somewhat prominent. In high-dose males, the incidence of hypertrophy of the anterior pituitary was significantly increased (45% compared with 2% of controls). A LOAEL of 322 mg/kg/day DEHP is derived from this study. Effects on the liver are described in detail in Section 4.1.2.7.1. No other toxic lesions were associated with compound administration.

The testicular effects were also investigated after dosing DEHP (purity not specified) at 2,000 mg/kg bw orally for 7 consecutive days to 13-week-old male Wistar rats (Saxena et al., 1985). Degeneration was observed in about 40% of the seminiferous tubules. Loss of succinic dehydrogenase, NADH-diaphorase and acid phosphatase activity and increase in adenosine triphosphatase, glucose-6-phosphate dehydrogenase and alkaline phosphatase activity were observed in treated rats. A LOAEL of 2,000 mg/kg/day DEHP is derived from this study.

The reversibility of the testicular effects was studied after oral administration of 2,000 mg/kg bw/day of DEHP (purity not specified) to young Crj:Wistar rats (35 days; 95-112 g (average 130 g)) for 14 days (Oishi, 1985). One day after the last administration, 10 dosed animals were sacrificed and compared with 10 rats fed control diet for additional 45 days without further administration of DEHP. Testicular morphology was characterised by a marked shrinkage of the seminiferous tubules, the germinal epithelium consisting of Sertoli cells, very few spermatogonia, and several multinucleated cells. The interstitial tissue and Leydig cells appeared normal. After a recovery period of 45 days after termination of DEHP administration, the majority of tubules showed lack of spermatogenesis, but some tubules had intact epithelium. The percentage of spermatogenic tubules in a representative cross section was 0 (total atrophy) or 12.8%. A LOAEL of 2,000 mg/kg/day DEHP is derived from this study.

In a 102 week-study, adult male Sprague-Dawley rats were exposed to DEHP via incorporation in the diet at dose levels of 0, 0.02, 0.2 or 2% (0, 7, 70 or 700 mg/kg bw/day) (Ganning et al., 1987, 1990) (see also Section 4.1.2.7.1). In all dose-groups, DEHP exerted a pronounced effect on the function of the testes after prolonged treatment, consisting of inhibition of spermatogenesis and general tubular atrophy. The LOAEL was 0.02% in the diet (7 mg/kg bw/day), the lowest dose administered. The study was, however, designed to study the effects of phthalates on the liver and the information on testicular effects is very limited. Therefore, the study results cannot be included in the risk assessment.

Mechanistic studies

Role of zinc, testosterone, protein content and vitamin B_{12}

Seven immature male Crj: Wistar rats (30 days, 75-90 g) per group were orally dosed with DEHP (2,000 mg/kg bw/day) for 0, 1, 3, 6 and 10 days (Oishi, 1986). Organ weights were significant decreased: testes by day 3; Seminal vesicle by day10; ventral prostrate by day 3. Testicular morphology was normal on day 1 but changes occurred for longer exposures. By day 3: number of spermatocytes and spermatids were decreased in some seminiferous tubules; day 6: active spermatogenesis was rarely found, seminiferous tubules contained necrotic debris and variable numbers of multinucleated gaint cells. By day 10, all seminiferous tubules had shrunken. Zinc concentration in the testes significantly decreased by by day 6 and 10, and by day 10 in the ventral prostrate. The zinc content was not affected in the seminal vesicle and serum. Specific activities of some zinc containing enzymes such as carbonic anhydrase, alcohol dehydrogenase and aldolase significantly decreased by day 10. The author concludes that several testicular cell-specific enzymes appear to be useful biochemical markers of testicular injury. However, these changes occurred after or simultaneously with massive histological or morphological changes rather than prior to such changes.

In a study comparable to a guideline study, groups of 48 male F344 rats were maintained on synthetic diets containing 2 ppm (low), 20 ppm (normal) or 200 ppm (high) zinc (Agarwal et al., 1986a). After one week of acclimation to the various diets, groups of 12 rats from each dietary regimen were gavaged for 13 consecutive days with 0, 330, 1,000 or 3,000 mg/kg bw of DEHP

(> 99% pure). Organ weights of testis, epididymis, prostate, and seminal vesicles were not affected by DEHP in rats at normal- and high zinc diet, but were significantly and dose-dependently reduced in rats on low-zinc diet. The combination of low-zinc diet plus 1,000 or 3,000 mg/kg bw of DEHP caused dose-dependent tubular degeneration and atrophy.

Seven young male Crj: Wistar rats (115 g) per group were orally dosed with DEHP (2,000 mg/kg bw/day) for 10 days (Oishi, 1986). Zinc sulphate (9 mg/kg) was co-administered intraperitoneally. DEHP caused a significant reduction in testes weight and testicular zinc concentration. Co-administration of zince did not significantly prevent the DEHP-induced effects.

The involvement of testosterone in the testicular atrophy caused by DEHP was examined by co-administration of testosterone (1 mg/kg bw) subcutaneously along with 2,000 mg/kg bw of DEHP (purity not specified) in groundnut oil to adult male Wistar rats (150-200 g) for 15 days (Parmar et al., 1987). Administration of DEHP was found to significantly reduce relative and absolute testes weights and the sperm count (approximately 75%), and also significantly increase the activity of GGT, LDH, and β-glucuronidase and to decrease the activity of SDH and acid phosphatase. Co-administration of testosterone seemed to normalise the testes weights, sperm count and the activity of testicular enzymes. The role of testosterone in testis toxicity of DEHP is not fully elucidated. Several reports refer to increased or decreased testosterone levels in plasma and testicular tissue.

A study of the influence of low protein diet on the testicular toxicity of DEHP was performed in adult male Wistar rats (150 g; 12 weeks old) (Tandon et al., 1992). One group of 12 animals received a synthetic diet containing 20% casein “normal protein diet”and the other 8% starch “low protein diet”. After 15 days of consumption, half of the rats in each group received 1,000 mg/kg bw of DEHP (purity not stated) in 0.2 ml groundnut oil orally for 15 days. The other half served as a control group. The group on “low protein diet” had a more severe response to DEHP compared with the group on the “normal protein diet” and their resective controls with regard to sperm count (75% see 30%), increase in the activity of beta-glucuronidase and gamma-glutamyl transpeptidase. The authors conclude that rats fed low-protein diet are more vulnerable to the toxic effect of DEHP.

A study of the influence of the vitamin B_{12} derivative adenosylcobalamin on testicular toxicity of DEHP was performed in young male Crj:Wistar rats (30 days; 86-100 g) (Oishi, 1994). Groups of 8 animals each were treatted for 7 days. DEHP (0 and 2,000 mg/kg bw/day) was given orally and co-administration of adenosylcobalamin or methylcobalamin, both 0.5 mg/kg, was administered intraperitoneally. DEHP significantly reduced body, testis and prostate weights, inhibited active spermatogenesis, reduced the activity of testicular specific lactae dehydrogenase, and decresed the levels of testicular zinc, magnisium and potassium. Co-administration of adenosylcobalamin, but not methylcobalamin, prevented the DEHP-induced effects.

Age-dependency

Gray and Butterworth (1980) found an age-dependent induction of testicular atrophy in rats, younger rats being more sensitive than older ones. Four-, 10-, and 15 week old Wistar rats were administered 2,800 mg/kg bw of DEHP (purity not specified) dissolved in corn oil by oral intubation for 10 days. In some experiments, testosterone propionate (200 µg/kg/day in corn oil) or FSH was given subcutaneously. The reversibility was studied in 4-week old rats at a dietary level of 2.0%. The testes were fixed in buffered formalin.

In 4-week old rats DEHP produced uniform seminiferous tubular atrophy, comprising loss of spermatids and spermatocytes. In 10-week old rats 5-50% of the tubules were atrophic; the testicular weight was not affected. A marked decrease of the testicular weight (% of control) was shown in 4-week-old rats. The weights of the seminal vesicles and ventral prostate were reduced in the 4- and 10- week old males. The testicular effects were found to be reversible within 12 weeks when treatment was stopped prior to puberty, but recovery was slower when treatment continued throughout puberty. Simultaneous administration of testosterone or FSH with DEHP did not affect the development of testicular atrophy, but prevented the reduction of accessory gland weights. The interstitial tissue appeared to be unaffected in all dosed animals. No effects were observed in 15-week-old rats.

To study the differing response between immature and mature male rats, Sjöberg et al. (1985c) carried out a series of experiments. Groups of 8 male Sprague-Dawley rats (25, 40 or 60 days old) were dosed with 0 or 1,000 mg/kg bw of DEHP in corn oil by gavage for 14 days. After sacrifice liver, testes, ventral prostate, and seminal vesicles were removed, cleaned from fat and weighed. The left testis and epididymis were fixed in Bouin's fluid for histopathological examination. The liver weight was significantly increased in all three age groups. The absolute testicular weight was significantly decreased; histopathological examination showed severe testicular damage in the 25-day-old rats, whereas the older animals were unaffected. In the youngest age group, there was a marked reduction in the number of germ cells, a high occurrence of degenerating cells, and a reduction of the tubular diameter. There also was a marked reduction in the number of spermatogonia.

A higher sensitivity in developing and sexually immature rats was observed by Arcadi et al. (1998) (see Section 4.1.2.10.4 for further details). Irreversible testicular effects were observed in pups exposed pre- and postnatally to DEHP in drinking water at 32.5 or 325 µl/litre (roughly corresponding to 3.0-3.5 mg/kg/day and 30-35 mg/kg/day, respectively). Only minor histological damage of the testes was observed in adult rats (vacuolisation of Sertoli cells accompanied by seminiferous tubules filled by cellular deposit in one out of four rats at 325 µl/litre.

To establish the compound or compounds responsible for the testicular damage after oral administration of DEHP, Sjöberg et al. (1986a) administered DEHP and five of its major metabolites (MEHP, 2-ethylhexanol and three identified metabolites (V, VI, or IX) of MEHP) for five days. Groups of 6 male Sprague-Dawley rats (35 days old at the start of the experiment) were given 2.7 mmol kg bw of DEHP (1,055 mg /kg bw) or one of the metabolites. A counting of degenerated cells per tubular cross section was carried out. No testicular damage was observed following oral doses of DEHP or 2-EH. The number of degenerated spermatocytes and spermatids was increased in animals which received MEHP; no such effects were seen in animals given the MEHP-derived metabolites.

In another study, Sjöberg et al. (1986b) also studied the age-dependent testis toxicity of DEHP (1,000 and 1,700 mg/kg bw in the diet for 14 days) in rats at 25, 40 and 60 days of age. Body weight gain was retarded in all dosed groups and testicular weight was markedly reduced in 25- and 40-day-old rats given 1,700 mg/kg bw. Severe testicular damage was shown for the 25-day- and 40-day-old rats at both dose levels. No changes were found in the 60-day-old rats. The authors propose that the difference in response to DEHP to male rats of different age may be due to a higher oral absorption of the DEHP-derived metabolite MEHP in younger animals (Sjöberg et al., 1985c) (see section on "Toxicokinetics").

Male Sprague-Dawley rats (4, 10, or 15 weeks of age, 8 animals per group) were used to study the age-dependent effects on male reproductive organs (Gray and Gangolli, 1986). The rats were given 2,800 mg/kg bw of DEHP (purity not specified) orally for 10 days. Administration to

4-week-old rats produced a marked reduction in absolute weights of the testes, seminal vesicles, and prostate. There was only a slight reduction in testis weight in 10-week-old rats but the seminal vesicle and prostate weights were significantly reduced. DEHP had no effect in 15-week-old rats. Histologically, the testes of the 4-week-old rats showed severe atrophy affecting virtually all the tubules. These were populated only by Sertoli cells, spermatogonia, and occasional primary spermatocytes. In the 10-week-old rats, these histological changes were evident in 5 to 50% of the tubules, the remainders appearing essentially normal. No histological abnormalities were seen in testes from the 15-week-old rats.

In the same study, the effects of MEHP on Sertoli cell function were studied in immature rats by measuring the secretion of seminiferous tubule fluid and androgen binding protein. A single dose of 1,000 mg/kg bw of MEHP reduced fluid and protein production to around 50% of the concurrent control group and to 25% after three repeated doses.

Dostal et al. (1988) studied the age-dependency of testicular effects (study comparable to a guideline study) in rats of different age. Oral doses of 0, 10, 100, 1,000 or 2,000 mg/kg bw of DEHP (> 99% pure) were given daily by gavage in corn oil for 5 days to Sprague-Dawley rats (7-10 animals per group) at 1, 2, 3, 6 and 12 weeks of age. Absolute and relative testis weights were significantly reduced at doses of 1,000 mg/kg bw/day in 1, 2, 3 and 6- week-old but not in 12-week-old rats compared to controls of the same age. Doses of 2,000 mg/kg bw/day were fatal to suckling rats and caused decreased relative testis weight but no lethality in 6- and 12-week-old rats. The number of Sertoli cell nuclei per tubule was reduced by 35% at 1,000 mg/kg in neonatal rats; two- and three-week old rats showed loss of spermatocytes but not of Sertoli cells. At 1,000 and 2,000 mg/kg also loss of spermatids and spermatocytes was shown in 6- and 12-week old rats.

To further understand the mechanisms responsible for the enhanced sensitivity of the testes of developing animals to DEHP, the activities of the testicular enzymes associated with spermatogenesis including LDH, GGT, SDH, β-glucuronidase, and acid phosphatase were studied in a similar study investigating the oral effect of DEHP on 25 day old male Wistar rats (Parmar et al., 1995). Doses of 0, 50, 100, 250 or 500 mg/kg bw of DEHP (purity not specified) in groundnut oil were given for 30 consecutive days to 6 male rats per dose group. There was an exposure-related and significant decrease of absolute and relative testicular weight at all dose levels. From 50 mg/kg also a dose-dependent and significant increase in the activities of LDH and GGT was noted while that of SDH decreased. β-glucuronidase increased at 250 or 500 mg DEHP/kg, while acid phosphatase decreased at the same dose levels. The administration also resulted in marked destructive changes in the advanced germ cell layers and marked degrees of vacuolar degeneration in the testes at 250 and 500 mg/kg bw. The significant alterations in the activities of SDH, LDH, and GGT occurred thus at much lower DEHP levels and prior to the histopathological changes. The Leydig cells and the fibroblasts appeared normal. A LOAEL for young rats of 50 mg/kg bw/day is derived from this study for effects on absolute and relative testies weight, and reduced testicular enzyme activities.

Mice

In a continuous breeding study, comparable to a guideline study and performed according to GLP principles (0, 0.01, 0.1 or 0.3% DEHP (> 99% pure) by weight in the feed corresponding to 0, 20, 200 or 600 mg/kg bw/day (Lamb et al., 1987) see also earlier in this section and Section 4.1.2.10.3. A diet of 0.3% DEHP caused an increased liver weight (both absolute and relative) and significantly reduced weights of the reproductive organs in parental animals of both sexes (testes, epididymis, prostate, and seminal vesicles in males and ovaries, oviducts, and uterus in

females). All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. In addition, this dose level also caused decreased sperm motility and sperm concentration and an increased incidence of abnormal sperm forms. DEHP did not significantly decrease body weight gain at 0.3% DEHP. At the highest dose level, 0.3% in the diet (600 mg/kg bw/day), no pairs were fertile. A NOAEL of 20 mg/kg/day DEHP is derived from this study.

In an oncogenicity study performed according to GLP principles, B6C3F1 mice (50 animals/sex/group) were given 0, 3,000 or 6,000 ppm of DEHP (> 99.5% pure) in the diet for 103 weeks (NTP, 1982) (see also Section 4.1.2.7.1 and 4.1.2.9.1). Mean daily ingestion of DEHP was calculated to 672 and 1,325 mg/kg bw for low- and high-dose males, respectively. The survival rate was unaffected. In 14% of the high-dose males bilateral seminiferous tubular degeneration and testicular atrophy were observed. This lesion was also found in one control male mouse and in two low-dose males. A NOAEL of 672 mg/kg/day DEHP is derived from this study.

In a study performed according to EPA guidelines and the principles of GLP, B6C3F1 mice (70-85 of each sex/dose group, about 6 weeks of age at the initiation of the study) were administered DEHP daily in the diet at concentrations of 0, 100, 500, 1,500 and 6,000 ppm for 104 weeks (0, 19.2, 98.5, 292.2 or 1,266 mg/kg/day) (Moore, 1997) (see also Section 4.1.2.7 and 4.1.2.9). One additional group (55 males) were administered 6,000 ppm DEHP for 78 weeks, followed by a 26-week recovery period. At 1,500 ppm, there was a significant decrease in testicular weight, with an increased incidence and severity of bilateral hypospermia and an associated increased incidence of immature/abnormal sperm forms and hypospermia in the epididymis. At the highest dose level, there was a statistically significant decrease in survival, treatment-related clinical signs and a significantly reduced body weight gain. In the recovery group, the effects of DEHP in the kidney and testis were at least partially reversible following cessation of exposure. The NOAEL for testicular effects in this study is 500 ppm corresponding to 98.5 mg/kg.

Mechanistic studies

Role of zinc

Two strains of mice (Jcl:ICR and Crj:CD-1, four weeks old), were fed diets containing 0, 0.1, 0.2, 0.4 or 0.8% (approximately 300, 600, 1,200 mg/kg bw/day)of DEHP (purity not specified) for two weeks (Oishi, 1993). In ICR-mice, testicular weights were unchanged by DEHP treatment at all concentrations when compared to controls. In CD-1 mice, testicular weights were significantly reduced from a dose level of 0.2%. The testicular zinc content was statistically significantly reduced in both strains at dose levels of 0.4 and 0.8%. Testicular activities of lactate dehydrogenase isoenzymes (LDH-X) were significantly reduced in CD-1 mice from a dose level of 0.2%, while a significant reduction of testicular LDH-X activity in ICR mice was observed only at a dose level of 0.8%. The primary metabolite, MEHP, was significantly increased in blood samples of CD-1 mice at 0.8% when compared to ICR mice suggesting that toxicokinetic differences may explain some of the shown strain differences in susceptibility to DEHP testicular toxicity.

Young (not specified) ICL:ICR male mice were fed a diet of 2% DEHP (approximately 3,000 mg/kg/day) for one week. Final body weight was reduced, and testes and liver weights significantly increased. Zinc and testosterone concentrations in the testes were significantly decreased by DEHP-treatment (Oishi and Hiraga, 198**0**).

Hamster

Four to six weeks old male hamsters were used to compare the testicular toxicity of DEHP in Dunkin-Hartely hamsters and young Sprague-Dawley rats (28-42 days) (Gray et al., 1982). DEHP (purity not specified) was dosed at 2,800 mg/kg bw in rats and at 4,200 mg/kg bw in hamsters for nine days. MEHP was also studied using a dose of 1,000 mg/kg bw for five days in rats and nine days in hamsters. In hamsters minor effects, occassional tubular arophy (2/6 animals) was obserevd. DEHP did not significantly alter the testicular zinc content. MEHP produced testicular damage. Also the authors concluded that MEHP did produce unequivocal testicular damage but suggested that the difference between DEHP and MEHP may be explained by rate-limiting conversion of DEHP to MEHP in the hamster intestine (see Section 4.1.2.1.5).

Ferrets

Mature albino ferrets (18 months: 1,150-1,850 g) were given 1%w/w DEHP in their diets for 14 months (Lake et al., 1976).Six and seven animals were used in the control and treated gruops, respectively. The mean daily intake of DEHP was 1,200 mg/kg/day, but owing to their seasonal fluctuation in body weight the daily DEHP intake ranged from 650 to 2,000 mg/kg bw/day.

Ferrets fed the control diet showed a seasonal fluctuation in body weight over a range of 450 g. However, the DEHP-treated group of animals initially exhibited a marked loss of body weight followed by a smaller seasonal variation over a range of 200 g. The terminal body weight of the DEHP-treated group was significantly reduced (870 ± 20 g) compared with controls (1,270 ± 100 g).

Testes from both control and DEHP-treated animals showed active spermatogenesis with spermatids or spermatozoa in the seminiferous tubules. However, almost complete absence of germinal epithelium was observed in 3/7 treated animals. Relative testes weights, but not absolute, were significantly increased in the treated- compared with control group.

A LOAEL of 1,200 mg/kg/day DEHP is derived from this study.

Rabbits

No relevant studies have been identified.

Marmosets

In a 13-week oral study performed according to GLP principles, mature male marmosets (4/sex/group, from 13 or 14 months of age) were given daily doses of 0 (corn oil), 100, 500 or 2,500 mg/kg DEHP (purity not specified) in corn oil (Kurata et al., 1995; 1996; 1998) (see also Section 4.1.2.6.4). The body weight gain was significantly suppressed in males administered 2,500 mg/kg. Dose-related decreases in spleen weight were observed in all dosed males. Other organ weights, including liver, testes, and pancreas, were not different from the control weights. In the DEHP dosed groups there was a significant rise in the total and free cholesterol and phospholipid levels in administration week 4. In week 13, only the total cholesterol value in the 500 mg/kg males was different from the control value.

A clear rise in blood testosterone and oestradiol concentrations in all groups, including controls, were concluded to be hormonal changes accompanying sexual maturity occurring at the age of about 12 months. CLEA Japan, Inc, the animal supplier, have communicated that male marmosets are sexually mature at around 7 months and mate at around 12 months (CLEA, 2000).

Other routes

Six intravenous infusions of DEHP (0, 5, 50 or 500 mg/kg bw) were given to 25-day- or 40-day-old rats (Sjöberg et al., 1985e). In Epon-embedded testicular materials from animals given the highest dose, some altered Sertoli cells (dilated cisternae of endoplasmic reticulum) were observed. No age-related testicular effects, similar to those found in the previous reported study (Sjöberg et al., 1985c), were observed.

Ten male albino rats (4 to 6 weeks old) were intraperitoneally injected either with DEHP (5 ml/kg bw; purity not specified) or with saline on days 1, 5 and 10 (Seth et al., 1976). On the 22nd day of the study, all animals were sacrificed. The activity of succinic dehydrogenase and ATPase in testes was significantly reduced, while that of beta-glucuronidase was increased. The testes of DEHP treated rats showed markedly thickened tunica albuginea. The degenerated tubules showed marked vacuolation of cytoplasm of spermatogonial cells and eccentric nuclei. Lumen of the tubules was filled with degenerated spermatids and eosinophilic material with entangled spermatocytes.

In vitro studies

Bell (1982) summarised a number of previous studies on the effect of DEHP dosing on synthesis of sterols and included in summary form some additional data. His conclusion was that DEHP significantly decreased sterol synthesis in the liver of male and female rats and male rabbits and in the adrenals and testes of rats. After placental transfer, a significant decrease of the sterol synthesis in the liver and brain of the pups was observed.

Grasso et al. (1993) studied the effects of DEHP and MEHP on cultured rat Sertoli cells. The Sertoli cells were obtained from Fischer 344 rats (13-82 days of age) and cultured in the presence of MEHP at concentrations ranging from 0.001 to 100 μM. MEHP was found to specifically reduce the ability of FSH to stimulate cAMP accumulation in rat Sertoli cells. This inhibition by MEHP of FSH-stimulated cAMP accumulation had a lag period of 6 hours and reached a maximal inhibition of 40-60% after 24-hours. Preincubation of Sertoli cells for 24 hours with 100 μM DEHP had no effect on FSH binding. The authors concluded that the ability of certain phthalate esters to disrupt the FSH-linked signal transduction pathway in primary Sertoli cell cultures by a mechanism, located at the cell membrane, is likely to be a part of the mechanism responsible for their testicular toxicity.

Metabolites of DEHP

The formation of the monoester is an important step in the intestinal absorption of the orally ingested phthalates. In pupertal and adult animals, the testicular toxicity of DEHP appears to be mediated by the monoester MEHP. Studies mainly focusing on this or other metabolites are therefore compiled separately.

Oral

Rats

Dalgaard et al. (2001) studied the effects of MEHP on 28-day old male rats, by looking at testicular morphology and apoptosis, and expression of some cellular markers (vimentin filaments, the androgen receptor, and a gene coding a Sertoli cell secretory product) 3, 6 or 12 hours (n=12) after a single oral dose of 400 mg/kg MEHP.

At 3-12 hours, vimentin filaments in Sertoli cells had collapsed, and the expression of the apoptos gene Caspase-3 was increased. However, there were no other indications of apoptosis as measured by DNA ladder analyses or tunel staining. The expression of TRPM-2 (coding a Sertoli cell secretory product) was transiently increased at 3 hours. At 3 hours there were no histological signs of toxicity, but at 6 and 12 hours the tubuli were disorganised and germ cells detached and sloughed into the lumen of the seminiferous tubules. The results support the Sertoli cells being early targets for MEHP toxicity.

The testicular toxicity of DEHP (> 98% pure) was studied in male Wistar rats (26 days old, 6 animals per group) after a single oral dose of 2,800 mg/kg bw (Teirlynck et al., 1988). In the same experiment, MEHP was given in doses of 400 or 800 mg/kg bw. The doses were selected in accordance to previous data showing that oral administration of 2,800 mg/kg bw of DEHP and 400 mg/kg bw of MEHP leads to similar MEHP plasma levels. Seven days after dosing the rats were killed and the testicular zinc concentration was measured. The severity of the histopathological lesions was graded on the basis of the percentage of seminiferous tubules affected. The diameter of the seminiferous tubules was also measured. The rats showed testicular atrophy 7 days after dosing, as indicated by a significant reduction in relative testicular weight. Histological examination revealed a "dose-dependent" increase in the number of atrophic seminiferous tubules with decreased diameters of the seminiferous tubules and loss of spermatids and spermatocytes,. The study suggests that MEHP is more toxic to the testes than DEHP. A significant reduction of the testicular zinc concentration was observed in DEHP treated rats and in rats given MEHP doses of 800 mg/kg bw, but not at doses of 400 mg/kg bw. The concentration of the follicle stimulating hormone (FSH) in serum was determined but no treatment-related alteration was observed. The authors suggest that the toxic effects of MEHP are not secondary to inhibition of pituitary gonadotropin secretion and that the absence of an elevation of FSH suggests that the function of the Sertoli cells is preserved.

Prepubertal male Fischer rats (28-day-old; number not stated) were given a single 2,000 mg/kg dose of MEHP (95% pure) in corn oil by gavage at a volume equal to 4 ml/kg (control rats received a similar volume of corn oil) to study the effect on germ cell apoptosis in testes (Richburg and Boekelheide, 1996). Preliminary experiments had also suggested that phthalates may cause alterations in the rat Sertoli cell cytoskeleton, particularly the intermediate filament vimentin. The rats were killed at 3, 6 and 12 hours after treatment. From each rat, one testis was rapidly frozen in liquid nitrogen and the other was cut in halves for immersion fixation in Bouin´s fixative and in neutral buffered formalin. Cryosections were stained with a monoclonal antibody to bovine vimentin. *In situ* Tunel staining was used to stain for DNA. The number of apoptotic germ cells was counted in 100 randomly selected seminiferous tubules of testis cross sections from each of four different rats. MEHP induced collapse of Sertoli cell vimentin filament 3 hours after MEHP-administration. Six and 12 hours after MEHP exposure, intense vimentin staining surrounding the nucleus was seen, suggesting that vimentin filament had collapsed toward the Sertoli cell nucleus. The incidence of apoptotic events observed in 100 seminiferous tubule cross sections of testes from each of four rats was counted and tabulated into categories. In control testes, 44.5% of the seminiferous tubule cross sections did not contain any apoptotic cells. However, 3 hours after MEHP treatment, the number of tubule cross sections with no incidence of apoptosis significantly increased to 63.3%. This shift was reflected by a significant decrease in the incidence of tubules containing 1-3 apoptotic cells per cross section at 3 hours. Cross sections of the seminiferous tubules from the 6- and 12-hours groups showed a dramatic increase in the number of apoptotic events as evident by the increased incidence of seminiferous tubules which contained high categories of apoptotic germ cells and a decrease in the incidence of seminiferous tubule cross sections that contained no apoptosis. The authors suggest that the MEHP-induced collapse in vimentin filaments may lead to alterations in germ

cell apoptosis by a disruption in contact-mediated communication between the Sertoli cells and germ cells and that the normal physiological incidence of germ cell apoptosis decreased as early as 3 hours after exposure to MEHP.

Four phthalate diesters, DEHP, DPP (di-n-pentyl phthalate), DOP (di-n-octyl phthalate), and DEP (diethyl phthalate) were investigated *in vivo* for effects on Leydig cell structure and function (Jones et al., 1993). The study was performed due to earlier study results indicating that communication and control exists between Sertoli and Leydig cells which appear to be of a paracrine nature. The corresponding monoesters were investigated *in vitro* (MEHP, MPP, MOP, and MEP). The *in vivo* study was performed by giving 2,000 mg/kg bw by oral gavage on two consecutive days to 3 male Wistar rats (6-8 weeks old, 200-300 g bw) per phthalate. The rats were sacrificed 24 hours after the final dose. Testicular tissues were studied by light and electron microscopy after glutaraldehyde perfusion fixation, Taab embedding and toluidine blue staining (a highly reliable technique in preparing testis tissue for identifying testicular toxicity). The *in vitro* study was performed with primary cultures of Leydig cells incubated with 1,000 µM monoester for 2 hours. Phthalate esters exerted a direct effect on Leydig cell structure and function as determined by testosterone output with correlation of the *in vitro* and *in vivo* effects of MEHP and DEHP, respectively. The changes observed *in vivo* were present in all animals in each group. Leydig cells stained more densely than other cell types, generally displaying an elongate profile often with thin lamellar processes. In Leydig cell cytoplasmatic ultrastructure, several subtle but highly significant alterations were produced. DEHP administration also resulted in slight rarefaction or vacuolation of a few Sertoli cells in seminiferous tubules, while treatment with DOP or DEP produced no change in seminiferous tubular structure or Leydig cell morphology. Exposure to DPP produced the most severe changes in Sertoli cells but no changes in Leydig cells. In the *in vitro* study, MEHP and MPP produced marked effects on structure and function including decreased LH-stimulated secretion of testosterone from Leydig cells incubated with MEHP while MOP caused decreased secretion and MEP was without effect. The results show that DEHP may exert a direct effect on Leydig cell structure and function and that DEHP and MEHP produce similar changes both *in vivo* and *in vitro* both in Leydig cells and in Sertoli cells. The authors concluded that a malfunction of Leydig cells likely affects the physiology of adjacent Sertoli cells. The authors also concluded that different phthalates may exert changes that are unique to one or common to both cell types.

In vitro studies

Exposure of rat seminiferous tubule cells in culture to 200 µM of either DEHP, 2-EH or MEHP resulted in a marked dose-related dissociation of germ cells from Sertoli cells only after exposure to MEHP (Gangolli, 1982). Preparations obtained from hamster cells did not respond to exposure to MEHP.

Sjöberg et al. (1986a) investigated the ability of DEHP, 2-EH, MEHP and metabolites V, VI, and IX to induce germ cell detachment from mixed primary cultures of Sertoli and germ cells. Only exposure to MEHP (10 µM for 24 hours or 1-200 µM for 48 hours) caused a significantly higher degree of germ cell detachment.

The effects of DEHP and MEHP on rat Sertoli cells *in vitro* was also studied by Grasso et al., (1993). It was shown that MEHP specifically reduced the ability of FSH to stimulate cyclic adenosine monophosphate (cAMP) accumulation in cultured Sertoli cells from rats, 13-82 days of age. This inhibition by MEHP of FSH-stimulated cAMP accumulation had a lag period of 6 hours and reached a maximal inhibition of 40-60% after 24 hours. Preincubation of Sertoli cells for 24 hours with 100 µM DEHP had no effect on FSH binding. The authors concluded that

the ability of certain phthalate esters to reduce FSH binding to Sertoli cell membranes is likely to be a part of the mechanism responsible for their testicular toxicity.

Cell cultures of Sertoli cells were also used to study lactate and pyruvate production after adding MEHP or DEHP (Moss et al., 1988). MEHP (0.1-200 μM) produced a concentration-dependent stimulation of lactate, but not pyruvate production over a 24-hour treatment period and an increase in the ratio of lactate/pyruvate concentration in the culture medium. DEHP had no such effects. The developing germ cells cannot utilise glucose to maintain ATP levels and are apparently dependent on a supply of lactate and pyruvate produced by Sertoli cells under control by FSH. The authors conclude that loss of germ cell in phthalate-induced testicular atrophy is not due to inhibition of energy substrate production by the Sertoli cells and that stimulation of lactate production may be a useful *in vitro* marker for phthalate esters that cause testicular injury.

Li et al. (1998) studied the effects of MEHP and DEHP on neonatal Sertoli cells and gonocytes (primitive spermatogonia) maintained in hormone- and serum-free coculture. They found that MEHP induced gonocyte detachment from the Sertoli cell monolayers in a time and dose-dependent manner. The cocultures of Sertoli cells and gonocytes were prepared from testes of 2-day-old male rat pups. Final concentrations of 0.01, 0.1, or 1.0 μM MEHP (≥ 99% pure) was added to the cocultures. DEHP (≥ 99% pure) was used as a negative control. At a dose of 0.1 μM MEHP, gonocytes rounded up and started to detach from cocultured Sertoli cells after 24 hour of exposure. At 1.0 μM, MEHP caused a rapid detachment of gonocytes detectable after 12 hours of exposure. No morphological changes were found in cultures treated with vehicle alone or with DEHP, added at a 10-fold higher concentration than the maximal dose of MEHP.

When cultures were labeled with BrdU (5-bromo-2'-deoxyuridine) few labeled cells could be found in the cultures treated with 1.0 μM MEHP compared to controls. No visually detectable increase in labeling could be observed in cultures simultaneously treated with FSH and 1.0 μM MEHP. Labeling indices in cultures treated with 0.1 or 1.0 μM MEHP were significantly lower than that in the vehicle-treated controls, reflecting decreases in Sertoli cell proliferation of 33.6 and 83.6%, respectively, over controls. The labeling indices of cultures treated with 10 μM MEHP was, however, significantly higher than that of the vehicle-treated controls. The study results show that MEHP directly targets the Sertoli cells and impairs their proliferation and that MEHP also may affect the interaction of gonocytes with Sertoli cells and/or target gonocytes directly. The findings also show that phthalate-induced changes in germ cell-Sertoli cell adhesion may occur during early postnatal development in rats.

The *in vitro* effects of a 24 hours exposure of a Leydig cell line to MEHP were studied by means of electron microscopy (EM) and by measuring progesterone production and cell viability (Dees et al., 2001). At a concentration of 1 μM MEHP, the first signs of toxicity appeared, as indicated by morphological changes involving nuclei (heterochromatin, euchromatin and large nucleoli), mitochondria (generally condensed, but some were swollen or had an abnormal form and contained degenerated christae) and presence of moderate numbers of lipid droplets. At 10 μM MEHP, the cell shape was affected (large and round to oval), SER was lost, large vacuoles and numerous lipid droplets were present in the cytoplasm, and some mitochondria were seen in close apposition to the lipid droplets. At 100 μM MEHP, the effects were more severe and some apoptotic cells were seen. A more general cell death was observed at 1-3 mM, as determined both by structure and a cell viability assay (MTT). Progesterone production was reduced in the 1-10 μM range (by approximately 50%), returned to normal values at 100 μM, and ceased when cell started to die. The authors discuss a mechanism where the mitochondria are the first targets of the toxicity, they then fuse with lipid droplets and degrade. The study conduct seems proper,

and the study pinpoints Leydig cells as potential sensitive targets for the DEHP-metabolite MEHP. However, the relevance for DEHP toxicity is not clear.

Lovekamp and Davis (2001) studied the *in vitro* effects of a 48 hours exposure period to 0-200 μM MEHP on primary rat granulosa cells. The authors find dose-dependent effects of MEHP on the levels of aromatase RNA, which is decreased as from exposure to 50 μM MEHP, and on the amount of aromatase protein and estradiol production (as from 100 μM MEHP). The relevance to DEHP toxicity is not clear.

4.1.2.10.2 Comparative studies

Mechanistic studies

Role of zinc

Curto and Thomas (1982) examined changes in testes and sex accessory weight as well as gonadal zinc in sexually mature rats and mice injected with various doses of DEHP or MEHP (purities not specified). Intraperitoneal and subcutaneous routes of administration were used to avoid hydrolyzation in the gastrointestinal tract and to exclude phthalate-induced reduction in the gastrointestinal absorption of zinc. Male Swiss-Webster mice (number not stated) received one of the following dose regimens: a) daily sc injections of 1, 5 or 10 mg/kg MEHP for 5 days; b) daily sc injections of 5, 10 or 20 mg/kg MEHP for 10 days; c) daily ip injections of 50 or 100 mg/kg MEHP or DEHP for 5 days; or d) alternate daily ip injections of 50 or 100 mg/kg MEHP or DEHP for 20 days (10 injections). Male Sprague-Dawley rats (number not specified) received daily ip injections of 50 or 100 mg/kg MEHP or DEHP for 20 days (10 injections). In mice, no significant alterations in testicular weight, seminal vesicle or anterior prostate weight or zinc levels occurred. Rats revealed significant reductions in both gonadal and prostate gland zinc. Rats injected with MEHP (50 mg/kg) showed a 37% decrease in prostate zinc; DEHP (100 mg/kg) caused a 33% decrease in prostatic zinc and a significant loss of testicular zinc (31%). The results indicated that the male rat is more sensitive to DEHP- or MEHP-induced effects on male gonads than the male mouse. It was also shown that sc or ip injected DEHP or MEHP caused gonadal zinc depletion, thus eliminating altered intestinal absorption as the cause for species differences.

4.1.2.10.3 Female reproductive toxicity

Oral

Rats

In a study comparable to a guideline study, regularly cycling Sprague-Dawley rats (6-9 in each study group) were dosed daily with DEHP (> 99% pure) at 2,000 mg/kg bw in corn oil by gavage for 1-12 days (Davis et al., 1994 a,b). Ovarian morphology and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and progesterone levels were analysed. DEHP treatment resulted in prolonged oestrus cycles compared to a control group. DEHP also suppressed or delayed ovulation by the first prooestrus/oestrous after the metoestrus-initiated dosing. Histopathological evaluation of the ovaries showed that 7 out of 10 DEHP-exposed rats had not ovulated by vaginal oestrous in contrast to 13 out of 13 control rats which ovulated by vaginal oestrous. Pre-ovulatory follicles were quantitatively smaller in DEHP-exposed rats than

in controls due to smaller granulosa cells. Suppressed serum oestradiol levels caused a secondary increase in FSH levels and did not stimulate the LH surge necessary for ovulation. According to the authors, these results suggest that DEHP-treatment causes hypo-oestrogenic anovulatory cycles and polycystic ovaries in adult female rats.

The effects of *in vivo* administered DEHP (1,500 mg/kg bw orally for 10 consecutive days) on *in vitro* ovarian steroid profiles in immature and cycling female rats have been studied by Laskey and Berman (1993). Groups of 20 and 21 mature female Sprague-Dawley rats were administered 0 or 1,500 mg DEHP/kg bw in corn oil for 10 days. On day 5 before dosing and daily during dosing, the stage of the oestrus cycle was determined for all animals. The day after the final dosing the animals were killed and ovaries, adrenals and serum were used to determine rates of steroid production. No ovary weight differences were noted in the control cycling animals or between the control and DEHP-treated rats. The alterations caused by DEHP in the *in vitro* ovarian steroidogenic profile were most apparent in rats during dioestrus and oestrous. In the DEHP dosed animals the incidence of animals in prooestrus was clearly reduced from day seven to day ten of dosing. In cultures of adrenals and serum no significant differences in rates of steroid production were observed. In the ovary cultures, di-oestrus rats dosed with DEHP had significantly higher testosterone and oestradiol production, and in rats in oestrus the oestradiol production was significantly lower in DEHP-dosed females. There were no significant differences in the steroid production of rats in prooestrus (only two dosed animals). The authors conclude that DEHP treatment alters the oestrus cycle and causes concentration changes of testosterone and oestradiol in rats in dioestrus.

In an oncogenicity study, performed according to EPA guidelines and in accordance with the principles of GLP, F-344 rats (70 males and females/group, about 6 weeks of age) were administered DEHP at dietary concentrations of 0, 100, 500, 2,500 or 12,500 ppm (0, 7.3, 36.1, 181.7 and 938.5 mg/kg/day in the females) for at least 104 weeks (Moore, 1996) (see Section 4.1.2.67 for details). An additional group was administered 12,500 ppm DEHP for 78 weeks, followed by a recovery period of 26 weeks.

There was a dose-related increased incidence of uterine mass at 2,500 and 12,500 ppm in females that died or were sacrified in extremis during the study, significant at the highest dose level. This was also found in females from the recovery group and in surviving animals from these dose groups at study termination.

Mice

Effects on fertility and reproduction in female mice have also been demonstrated. In a continuous breeding study, comparable to a guideline study and performed according to GLP principles (0, 0.01, 0.1 or 0.3% DEHP (>99% pure) by weight in the feed corresponding to 0, 20, 200 or 600 mg/kg bw/day) (Lamb et al., 1987) (see also Section 4.1.2.10.1). No treatment-related clinical signs of toxicity were found during the breeding phase of the study. One male (0.1%) and two females (0.3%) died. DEHP did not significantly decrease feed consumption and body weight gain in the high-dose females. Exposure to 0.1 and 0.3% DEHP in the diet produced dose-dependent and significant decreases in fertility (100, 100, 74, and 0%, respectively) and in the number and proportion of pups born alive (0.98, 0.99, 0.80, -). A dosage of 0.3% DEHP (equivalent to 600 mg/kg bw/day) caused an increased liver weight (both absolute and relative) and significantly reduced weights of ovaries and oviducts and uterus in females. The NOAEL was 0.01% in this study, equivalent to 20 mg/kg bw/day.

In a complementary crossover mating trial, females given 0.3% DEHP in the diet were mated to undosed control males. None of the females were able to produce pups: the fertility index was 0 (0/16) compared to 90% of the control group (18/20).

In two-generation study, DEHP was given in the diet at 0.0, 0.01, 0.025, and 0.05% (equivalent to 0, 19, 48, and 95 mg/kg bw, respectively) to CD-1 mice (NTIS, 1988). DEHP treatment did not affect the number of implantation sites per dam, the percent fertile matings, the pregnancies with live litters on pregnancy day 1, or the percent viable litters through gestation to postnatal day 4.

Other routes

The effect on the ovary of DEHP given by intraperitoneal administration (4,900 mg/kg bw on day 1, 5 and 10) was studied in 20 prepubertal female albino rats (Seth et al., 1976). On administration day 22, the animals were killed. One ovary from each animal was used for enzyme determinations and the other for histopathological studies. The activity of succinate dehydrogenase and ATPase was significantly reduced, while that of beta-glucuronidase was increased in the treated animals when compared with controls. No histopathological changes were seen in this study.

Male and female mice were subcutaneously administered 1-100 ml/kg bw of DEHP on day 1, 5 and 10 and assessed at day 21 for reproductive performance (Agarwal et al., 1989). A dose-dependent reduction in the incidence of pregnancy was seen from the 1 ml-dose level. Ovarian weights were not decreased by the DEHP-treatment; ovaries exhibited, however, histological injuries at lower dose level than the testes, but unlike testes the effect was not dose-related. Biochemical changes (significantly decreased activity of ATPase and significantly increased activity of lysosomal enzymes as Rnase, DNase, β-glucuronidase and acid phosphatase) were dose-related for ovaries and testes.

In vitro studies

The effects of MEHP on granulosa cell function were studied *in vitro* (Treinen and Heindel, 1992). It was shown that MEHP inhibited FSH- but not forskolin-, isoproterenol-, or cholera toxin-stimulated granulosa cell cAMP accumulation *in vitro*. MEHP also inhibited FSH-stimulated progesterone production, a cAMP-dependent process. Similar to MEHP, the protein kinase C activator (TPA) has been shown to inhibit rat granulosa cell cAMP accumulation in a FSH specific manner, and decrease FSH-stimulated progesterone production. According to the authors, these data indicate that the inhibitory effects of MEHP on granulosa cell function are independent of phorbol ester-sensitive PKC activation.

4.1.2.10.4 Developmental studies

Numerous studies have shown that DEHP is embryotoxic in rats at doses close to maternally toxic dose levels. In mice, several studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those producing observable evidence of toxicity to the dams.

Inhalation

Rats

Twenty-five pregnant Wistar rats per dose group were used to study the teratogenicity of DEHP by inhalation as liquid aerosol at dose levels of 0, 0.01, 0.05 or 0.3 mg/litre (0, 10, 50 or 300 mg/m^3) (Merkle et al., 1988). The particle MMAD was < 1.2 ± 7.3, ± 16.8 and ± 5.8 μm for the low, middle and high dose group, respectively. The study was performed according to OECD Guideline 414 and GLP principles. The dams were exposed by head-nose exposure for 6 hours per day from gestation day 6 through 15 (the period of male sexual differentiation between days 16-19 is not included in this study). Twenty rats per group were sacrificed on day 20 of pregnancy and five rats per group were allowed to litter. The offspring was raised and observed for postnatal signs of toxicity. In a range-finding study, "exposure-related" peroxisome proliferation was observed in dams from 200 to 1,000 mg/m^3. The number of live foetuses/dam was slightly, but statistically significantly decreased in the 50 mg/m^3 group and the percentage of resorptions/dam was elevated. These effcets, however, were not seen at the next dose level. That effects were only seen in the middle dose group may reflect the large standard deviation of the particle MMAD. The effects reported were not regarded as exposure related, since no dose dependency was observed. The number of corpora lutea, uterine weights, body weights, living and death implants, early and late resorptions, dead foetuses, pre- and post-implantation losses were unchanged compared to controls. The validity of this study is questioned as the systemic dose was not determined. By comparison with another inhalation dose study there may be problems with delivering the expected dose of DEHP (see Section 4.1.2.6.23 and Klimisch et al., 1992). Hence, this study is considered inadequate for use in risk characterisation.

Oral

Rats

Two multi-generation studies (Schilling et al., 2001 and Wolfe et al., 2003) in rats, that give important information on development as well, are described in Section 4.1.2.10.10. Based on Wolfe et al. (2003) a NOAEL of 4.8 mg/kg/day for developmental effects on the testis is deduced.

Arcadi et al. (1998) exposed female Long-Evans rats (12 rats/dose group) daily to drinking water containing DEHP at 32.5 or 325 μl/litre from day 1 of pregnancy to day 21 after the delivery. The water intake was roughly calculated to correspond to 3.0-3.5 and 30-35 mg/kg DEHP/day during pregancy; during suckling this value was increased by at least 30%, due to increased water intake. At different time after delivery (21, 28, 35, 42 and 56 days) eight pups/group were sacrificed. Pup body weight gain and kidney, liver and testis weights were measured. (The method for preparing testicular tissue included Zenker´s fluid fixation, paraffin embedding and haematoxylin-eosin staining.) Plasma levels of DEHP and histopathology of the kidneys, liver, and testes were also studied. Female pups were used for behavioural assessment 30 days after birth in the "beam walking" test, designed to assess the locomotor activity by employing a learned avoidance test.

Pregnancy rate, body weight gain and gross appearance in the dams were not affected by the treatment. No further maternal toxicity data are available. Perinatal exposure produced no significant changes in body weight gain in the pups. A statistically significant reduction in kidney weight (absolute and relative) was observed at both dose levels, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis,

dilation of renal tubuli and light fibrosis) between week 0 and 4 of age. The alterations were less pronounced at week 8. The increased liver weight was not dose related and was therefore considered not to be related to the exposure level.

A highly significant and dose-dependent reduced testicular weight (absolute and relative) was observed and did not appear to reduce with growth. The perinatal exposure caused severe histological damage to the testes. At 21 and 28 days of age, there was a gross disorganisation of the seminiferous tubular structure, detachment of the spermatogonial cells from basal membrane, and absence of spermatocytes in both exposure groups. At the end of the observation period, at 56 days, there were still severe histopathological changes in the testes of pups. Low-dose rats exhibited only a few elongated spermatids in tubules showing a pervious lumen. In high-dose animals the histological picture included a generalized disorganization of the tubular epithelium, with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa, and with the tubular lumen filled with cellular deposits.

Female pups exposed perinatally to 325 µl/litre of DEHP showed a significantly increased time necessary to perform the beam walking test indicating a behavioural effect expressed as reduced locomotor activity.

A LOAEL of around 3.5 mg/kg bw/day is derived from this study.

Moore et al. (2001) studied the effects of DEHP (0, 375, 750 or 1,500 mg/kg and day, by gavage) on male reproductive system development and sexual behaviour in Sprague-Dawley rats (n = 5-8/group). The exposure started at gestation day 3, ended at postnatal day 21, and male pups were examined at PND 21, 63 and 105. Numerous effects, including those normally observed after high doses of DEHP, such as malformations and reduced weights of organs related to the male sexual system, were observed in the pups at the highest doses. The lowest dose was a LOAEL (375 mg/kg and day), with findings of adverse effects on aerola and nipple retention, as well as testis and anterior prostate weight (reductions). Although not statistically significant, there were indications of effects on sexual behaviour at PND 105 in all dose groups (inactivity in 3 of 7 low dose males when kept together with females).

Parks et al. (2000) studied the effects of DEHP (750 mg/kg and day, by gavage) on male reproductive parameters in Sprague-Dawley rats. The exposure started at gestational day 14 and ended at postnatal day 3. Four-five dams were killed at gestation day (GD) 17, 18, 20 and at postnatal day (PND) 2, with 11-32 male pups examined at each timepoint.

Ex vivo testicular production of testosterone, testicular content of testosterone, and whole-body testosterone concentration were significantly reduced at all time points, with maximal effects at GD 20 (e.g. a 90% reduction in ex vivo testicular production of testosterone). Anogenital distance (PND 2) as well as testicular weight (GD 20 and PND 2) were also reduced. Histopathological examination of the PND 2-testes (fixed and sectioned (1µm) using EM techniques, but studied by light microscopy) revealed increased numbers of Leydig cell hyperplasias and of multinucleated germ cells. Leydig cell (LC) hyperplasia was supported by an increased staining of the LC-specific enzyme 3β-HSD. There is no mentioning of Sertoli cells in the histopathology section. The study shows pronounced effects of DEHP on male fetus and offspring, characterised by a decreased production of testosterone, Leydig cell hyperplasia, and formation of multinucleated germ cells, with the only dose studied obviously being an effect level (750 mg/kg and day).

In a study performed according to OECD Guideline 414 and GLP principles, DEHP was tested for its prenatal toxicity in Wistar rats (BASF, 1995; Hellwig et al., 1997). DEHP (99.8% pure) was administered as an oily solution to 9-10 pregnant female rats/group by stomach tube at doses

of 40, 200 or 1,000 mg/kg bw on day 6 through 15 of gestation. On day 20 of pregnancy, all females were sacrificed and assessed by gross pathology.

Maternal toxicity at 1,000 mg/kg bw was reported: Slightly reduced maternal food consumption was noted. Reduced uterus weight was assessed as to be associated with the high embryolethality (see below). The corrected body weight gain did not show any differences of biological relevance. Statistically increased relative kidney and liver weights was obserevd

Developmental toxicity at 1,000 mg/kg bw: Severe developmental effects were observed: statistically significantly increased implantation loss (about 40%). There also was a statistically significant lower number of live foetuses/dam, decreased foetal body weights, a drastically increased incidence of of external, soft tissue, and skeletal malformed foetuses/litter (in total approximately 70% of the foetuses/litter), predominantly of the tail, brain, urinary tract, gonads, vertebral column, and sternum. There also were an increased percentage of foetuses/litter with soft tissue and skeletal variations and skeletal retardations. The NOAEL for maternal and developmental toxicity was 200 mg/kg bw/day.

Gray et al. (1999) investigated the reproductive effects of ten known or suspected anti-androgens, including flutamide, Vinclozolin, dibuthyl phthalate (DBP) and DEHP.

Eight pregnant Sprague Dawley dams were administered DEHP (750 mg/kg bw/day; > 99% pure) in corn oil by gavage from gestation day 14 to day 3 of lactation (see also the section on endocrine activity, 4.1.2.10.8). The male offspring was examined for abnormalities (retained nipples, cleft phallus, vaginal pouch, and hypospadias). The animals were also examined internally (ectopic or atrophic testes, agenesis of the gubernaculum, epididymides, sex accessory glands, and ventral prostate, epididymal granulomas, hydronephrosis, and enlarged bladder with stones). Weights measured included body, pituitary, adrenal, kidney, liver, ventral prostate, seminal vesicle (with coagulating gland and fluid), testis, and epididymis. Gonads and sex accessory tissues were examined microscopically.

DEHP was considerably more toxic than was DBP to the reproductive system of the male offspring. The gestational and lactational exposure induced a statistically significantly increased incidence of both reproductive and nonreproductive malformations including decreased anogenital distance, areolas (88%), hypospadias (67%), vaginal pouch (45%), ventral prostate agenesis (14%), testicular and epididymal atrophy or agenesis (90%), and retained nipples in examined pups. In addition, several 8-day old pups displayed haemorrhagic testes by gross examination. In adult offspring (5 months old) the weight of the gonads, accessory sex organs, and the Levator ani-bulbocavernosus were statistically significantly decreased.

Gray and coworkers found that the chemicals investigated could be clustered into three or four separate groups, based on the resulting profiles of reproductive effects. DBP and DEHP induced a higher incidence of testicular and epididymal abnormalities, including atrophy and agenesis, which is not generally found with flutamide or Vinclozolin even at high dose levels. A LOAEL of 750 mg/kg bw/day is derived from this study.

In a study comparable to a guideline study and performed according to GLP principles, dietary levels of 0, 0.5, 1.0, 1.5 or 2% of DEHP (no information on mg/kg bw/day is given) were given to groups of F344/CrlBr rats (34-25) throughout gestation (days 0-20) (NTIS, 1984; Tyl et al., 1988). The rats were sacrificed on day 20.

Food intake was significantly decreased at all dose levels. Reduced maternal body weight gain and increased absolute and relative liver weights were observed at a dietary level of 1.0%. Reduced foetal body weights per litter were observed at the same dietary level. There were no

treatment-related differences in the number of corpora lutea or implantation sites per dam, nor in the percent preimplantation loss. At a dietary level of 2% the number and percent of resorptions, nonlive and affected implants per litter were significantly increased and the number of live foetuses per litter was significantly decreased. Mean foetal body weight was significantly reduced at all dose levels. The number and percentage of malformed foetuses per litter was not significantly different from control. The NOAEL for maternal and developmental toxicity was 0.5% DEHP (approximately 357 mg/kg bw/day).

DEHP (0, 333, 500, 750 and 1,125 mg/kg bw) was administered by gavage to Fischer-344 rats on gestational days 6-15 (Narotsky et al., 1995). In a parallel study the interaction of trichloroethylene and heptachlor with developmental toxicity of DEHP was analysed in a $5 \cdot 5 \cdot 5$ full-factorial design. The only developmental effects observed with DEHP alone were a significant delay in parturition and micro-/anophthalmia at 750 and 1,125 mg/kg bw. Synergistic developmental toxicity was demonstrated for several endpoints such as DEHP-heptachlor for maternal mortality, DEHP and trichloroethylene for maternal weight gain on gestational day 6-8, full-litter resorption, prenatal loss, and pup weight on postnatal day 6. Heptachlor potentiated the effects for full-litter resorption and prenatal loss. The DEHP-heptachlor interaction was antagonistic for several endpoints, but this was perhaps reflecting a "ceiling effect" of combining already high responses as suggested by the authors. The main effects of DEHP-trichloroethylene included a dose-related, increased incidence of microphthalmia and anophthalmia (pups with eye defects, $2.8\% \pm 2.1$ and $4.3\% \pm 3.4$ at 750 and 1,125 mg/kg bw, respectively, versus 0% in controls). No interaction was shown for this endpoint.

Srivastava et al. (1989) dosed groups of 21 pregnant albino rats (strain not specified) on day 6-15 of gestation with 0 or 1,000 mg DEHP/kg bw by gavage. On day 20 of gestation all pregnant rats were killed and seven litters from each group were used for standard teratology studies, the remaining 14 litters were used for a study of liver enzyme activities and determination of DEHP in liver tissue. There was no significant difference in the number of total live foetuses between control and treated animals. No gross or skeletal abnormalities were observed in the foetuses of the control or DEHP-exposed animals (no data were shown). Significant amounts of DEHP were, however, found in foetal livers and foetal relative liver weights were increased, whereas the activity of mitochondrial succinate dehydrogenase, ATPase, malate dehydrogenase and cytochrome c oxidase was decreased. The authors concluded that maternal exposure to DEHP during pregnancy could adversely affect the foetal livers. These results also indicate that DEHP can cross the placental barrier.

The effects on the testicular development in the offspring exposed to DEHP in utero were studied by Tandon et al. (1991). Groups of six pregnant rats were given vehicle (ground nut oil) or DEHP (1,000 mg/kg bw/day; purity not specified) by gavage, during the entire gestation period. Birth weight of all pups and body weight gain of two randomly selected male pups from each litter were recorded at day 7, 15, 31, 61 and 91 days of age. Absolute and relative testes weights were significantly reduced at day 31, but normalised at day 61 and 91. The offspring of rats exposed to DEHP during the gestational period exhibited a significant increase in the activities of testicular lactate dehydrogenase (LDH) and gamma-glutamyl transpeptidase and a decrease in sorbitol dehydrogenase at the age of 31 days, which was persistent up to the age of 61 days. The concentration of epididymal spermatozoa was significantly reduced day 91, the only day it was measured (5.04 ± 0.24 million in DEHP-treated versus 6.48 ± 0.35 in controls).

Results from a recently performed 2-generation range finding study in Wistar rats indicate effects on fertility and developmental toxicity (see also Section 4.1.2.10.1) (Schilling et al., 1999). The study was performed according to current guidelines and in conformity with GLP. Wistar rats (F0 generation = 10 rats/sex) were exposed to dietary levels of 0, 1,000, 3,000 or

9,000 ppm of DEHP (corresponding to approximately 0, 110, 339 or 1,060 mg/kg bw/day). F1 pups were raised and mated to produce a F2 generation, sacrificed 2 days after birth.

At 1,000 ppm, a significantly increased mean absolute liver weight was noted in F0 females. At the next higher dose level, 3,000 ppm, the mean relative liver weight was significantly increased in both males and females. No treatment related histopathological changes were, however, noted.

The food consumption, body weight, and body weight gain were significantly reduced in F0 females during premating, gestation, and lactation at 9,000 ppm. (The effects on males are described in more detail in Section 4.1.2.10.1) The post implantation loss was significantly increased (31% versus 10% in the controls) at this dose level. The total number of delivered F1 pups, the mean number of delivered pups per dam, and the survival on post partum day 0 and 4 were significantly reduced. The mean body weights were reduced until weaning in both male and female pups and the body weight gain was retarded. The incidence of areolas/nipple anlagen was increased in high-dose F1 pups (29 out of 35, 83%). The sexual maturation was delayed in both female and male pups based on vaginal opening and preputial separation, respectively. Histopathology showed a treatment related loss of spermatocytes in male F1 pups (in two out of ten at 3,000 ppm and seven out of nine at 9,000 ppm).

In F1 parental animals deaths (3/9 males and 2/9 females) occurred at 9,000 ppm in the premating phase, and initially also reduced food consumption, reduced mean body weights in both sexes, and reduced body weight gain in females. At necropsy aspermia (2/6), missing seminal vesicle (1/6), and areolas/nipple anlagen (1/6) were also noted. The total number of liveborn F2 pups was reduced from 3,000 ppm, significant at 9,000 ppm, and, at this dose level, the mean number of delivered pups/dam (66%; statistically significant) was reduced. The viability index was not calculated, since the pups were killed on postmortem day two. The anogenital distance was reduced in the F2 male pups.

The effects found in F1 parental males indicate that DEHP exerts a specific action on male genital organs such as the testicle and the epididymis, when males are exposed during early development. This is strengthened by the fact that female interior organs were unaffected.

However, concerning testicular effects in developing male pups only one testicle per litter was studied histopathologically in F1 pups and none of the F2 pups. F1 pups were culled at day 21 and neither undescended testes nor hypospadia were investigated. Neither is there any information on effects on Sertoli cells in F1 parental male rats in this range finding study as is seen in several other studies presented above (Gray and Butterworth, 1980; Sjöberg et al., 1985c; Sjöberg et al., 1986a,b; Gray and Gangolli, 1986; Dostal et al., 1988; Poon et al., 1997; Arcadi et al., 1998). However, according to preliminary data from the following main study presented by the Industry (ECBI/37/99 – Add.10), Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1,000 ppm.

In a study, presented only in abstract form, resorptions (16.4%) and malformations such as hydronephrosis and cardiovascular, tail and limb defects in 20.6% of survivors were reported in Wistar rats administered DEHP (25 mmol/kg, equivalent to 9,750 mg/kg bw) on day 12 of gestation (Ritter et al., 1987).

Mice

In a continuous breeding study, comparable to a guideline study and performed according to GLP principles, DEHP (> 99% pure) was given to CD-1 mice (20 animals of each sex per dose group and 40 control animals of each sex) at dietary levels of 0, 0.01, 0.1, or 0.3% (equivalent to 0, 20, 200, or 600 mg/kg bw/day, respectively) (Lamb et al., 1987) (see also Section 4.1. 2.10.1).

Both male and female mice were exposed during a 7-day premating period and were then randomly grouped as mating pairs. The dosing continued during the 98 day cohabitation period and thereafter for 21 days during which final litters were delivered and kept for at least 21 days. Reproductive function was evaluated by measuring the number of litters per breeding pair, number of pups per litter, proportion of pups born alive, and mean pup weight.

Exposure to 0.1% DEHP produced a dose-dependent and significant decrease in the number of litters as well as the number and proportion of pups born alive. No pairs were fertile at 0.3%. At a diet of 0.3% DEHP caused an increased liver weight (both absolute and relative) and significantly reduced weights of the reproductive organs in parental animals of both sexes (testes, epididymis, prostate, and seminal vesicles in males and ovaries, oviducts, and uterus in females). All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. In addition, this dose level also caused decreased sperm motility and sperm concentration and an increased incidence of abnormal sperm forms. DEHP did not significantly decrease body weight gain in the high-dose group.

A crossover mating trial conducted with F_0 mice showed a decrease in fertility both for treated males and for treated females, with a complete loss of fertility in the females. Four litters out of twenty were born to treated males mated to control females; in addition, the proportion of pups born alive was decreased. No pups were born when dosed females were mated to control males. The NOAEL for maternal and developmental toxicity was equivalent to 600 and 20 mg/kg bw/per day, respectively.

In a study comparable to a guideline study and performed according to GLP principles, dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% of DEHP (0, 44, 91, 190.6 or 292.5 mg/kg bw/day; > 99% pure) were administered to groups of 1-CR outbred mice (30-31 per group) throughout gestation (days 0-17) (NTIS, 1984; Tyl et al., 1988). Maternal toxicity, indicated by reduced maternal body weight gain, was noted in the two highest dose groups, mainly due to reduced gravid uterine weight. There were no treatment-related effects on the number of corpora lutea, implantation sites per dam, the percent pre-implantation loss, and sex ratio of live pups. The number and percent of resorptions, late foetal deaths, and dead and malformed foetuses were all significantly increased from 0.1%. Foetal weight and the number of live foetuses per litter was significantly reduced from the same dose level. Both the percentage of foetuses with malformations and the percentage of malformed foetuses per litter was significantly increased from 0.05%. The observed external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted, or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. The NOAEL for maternal toxicity was concluded to be 0.05% (91 mg/kg bw/day) and for developmental toxicity 0.025% (44 mg/kg bw/day).

In a dietary 2-generation study (comparable to a guideline study and performed according to GLP principles) in CD-1 mice, DEHP was given in the diet at 0.0, 0.01, 0.025, and 0.05% (equivalent to 0, 19, 48 and 95 mg/kg bw, respectively) to CD-1 mice (NTIS, 1988). DEHP treatment did not affect the number of implantation sites per dam, the percent fertile matings, the pregnancies with live litters on pregnancy day 1, or the percent viable litters through gestation to postnatal day 4.

The F1 generation was mated within dose groups at sexual maturity and F2-offsprings were evaluated for viability and growth at postnatal day 4. For F1-litters, the percentage of prenatal mortality was increased at the high dose (9% versus 26.4%). During the neo-natal period, the percent of viable pups was significantly decreased at 0.05% DEHP. No other effects of DEHP

were observed upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testes decent or vaginal opening, or spontaneous locomotor activity) on postnatal days 14, 21 or 50/day. Treatment-related lesions were not observed in the dams and no maternal LOAEL was established. The NOAEL for parental toxicity and for F2-offspring was 0.05% DEHP (95 mg/kg bw/day), the highest dose tested. The LOAEL for F1 offspring was 0.05% (95 mg/kg bw/day) (NTIS, 1988).

In two other oral studies in mice, doses of 0.05 to 30 ml/kg or 0, 250, 500 or 2,000 mg/kg bw elicited a wide range of malformations affecting particularly the skeletal system at doses from 0.1 ml /kg bw (about 98 mg/kg bw) (Nakamura et al., 1979; Tomita et al., 1982b). The developmental LD_{50} and the maximum non-lethal dose of DEHP after single oral administration was 592 mg/kg bw and 64 mg/kg bw (extrapolated dose), respectively.

DEHP was given to female ICR mice (8 to 16 weeks old) at dietary levels of 0, 0.05, 0.1, 0.2, 0.4 or 1.0% (equivalent to 0, 70, 190, 400, 830 and 2,200 mg/kg bw, purity not specified) from day 1 to 18 of gestation (Shiota and Nishimura, 1982). On day 18 the animals were killed. The average weight of live foetuses was decreased and the incidence of malformed foetuses was significantly higher from 400 mg/kg bw. The most common malformations were neural tube defects (exencephaly and spina bifida), malformed tail, gastroschisis and club foot. The NOAEL for maternal and developmental toxicity was 70 mg/kg bw of DEHP.

Huntingdon (1997) performed a GLP study of the embryo-foetal toxicity in the CD-1 mice by oral gavage administration. Doses of 0, 40, 200 or 1,000 mg DEHP/kg bw/day were administered to groups of 15 pregnant mice from day 6 to 15 of gestation. A control group of 30 pregnant mice received a vehicle (0.5% carboxymethylcellulose containing 0.1% Tween 80). Litter parameters following necropsy of the females on day 17 of gestation revealed low numbers of viable young, high numbers of resorptions, and a greater extent of post-implantation loss for females given 1,000 mg/kg bw/day than in the control group. Cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney anomalies were observed. At 200 mg/kg bw/day, there was a slightly higher incidence of foetuses with intra-muscular or nasal haemorrhage or dilated orbital sinuses. There also was a small number of foetuses with anomalous innominate or azygous blood vessels. From this study a NOAEL of 200 mg/kg bw/day can be derived for maternal toxicity and a NOAEL of 40 mg/kg bw/day for developmental toxicity.

In a study designed to identify days of gestation particularly sensitive to DEHP, groups of 3-8 female ddY-Slc(SPF) mice (7-8 weeks old) were given 0, 1.0, 2.5, 5, 7.5, 10 or 30 ml/kg bw of DEHP (> 99% pure and corresponding to 1/1, 1/3, 1/4, 1/6, 1/12 or 1/30 of the acute oral LD_{50}) by gavage on day 6, 7, 8, 9 or 10 of gestation (Yagi et al., 1980). Ethylurethane was given intraperitoneally to control mice. The average body weights of the foetuses were decreased at all dose levels regardless of the day of maternal exposure. The number of resorptions was increased largely dependent on the dose and particularly on the day of dosing. A high and dose-related increase was observed in animals dosed on days 7 and 8 of gestation at all dose levels tested. Doses of 5 or 10 ml/kg bw given on day 7 led to 100% fatality of all foetuses. The incidence of foetal deaths was considerably less when DEHP was administered on day 9 or 10 of gestation. Dose levels of 2.5 or 7.5 ml/kg DEHP given to mice on day 7 or 8 of gestation induced a high incidence of gross and skeletal abnormalities including encephaly, open eyelid, and club foot. There is no information on maternal toxicity.

No maternal or embryo-foetotoxicity were observed in eight female mice given 4,000 mg DEHP/kg bw by gavage on days 7, 8 and 9 of gestation (Shiota and Mima, 1985).

Rabbits

No developmental studies have been performed in rabbits given DEHP. Study results have only been obtained from a study in rabbits given MEHP intravenously (see below).

4.1.2.10.5 Post-natal study

Oral

Rats

Two combined pre- and post-natal exposure studies conducted by Arcadi et al., (1998) and Gray et al. (1999) have been presented above under Section 4.1.2.10.1: A LOAEL ≈ 3.5 mg/kg bw/day and 750 mg/kg bw/day was derived for each of these studies, respectively

Parmar et al. (1985) exposed dams (5 per group) to 0 or 2,000 mg DEHP/kg bw. Litters of seven pups were dosed with DEHP through mothers milk through the lactation period (from parturition to day 21). Pup body weights were recorded with five days interval and at sacrifice on day 21. Pooled liver homogenates were prepared for an assay of the activities of arylhydrocarbon hydroxylase, aniline hydroxylase and ethylmorphine N-demethylase, and concentration of cytochrome P-450. The body weight of the DEHP treated pups was lower than that of the control group throughout the whole period. Absolute liver weight was significantly decreased in the DEHP treated pups; relative liver weight was similar in the two groups. All four biochemical parameters showed significant decreases in the DEHP treated pups relative to control. In the livers of the pups, a concentration of 25.7 µg DEHP/g was found. The authors conclude that DEHP and its metabolites can be transferred to pups via mothers milk in concentrations sufficient to cause toxicity.

Female Sprague-Dawley rats were given 5 oral doses of 2,000 mg/kg bw/day of DEHP (> 99% pure) in corn oil by gavage on days 2-6, 6-10 or 14-18 of lactation (Dostal et al., 1987a). The rats were sacrificed 24 hours after the last dose. The body weights of lactating rats and of their suckling pups were significantly reduced in all treatment intervals. Food consumption was reduced in the mothers dosed on days 14-18. Relative liver weights were increased in the lactating dams at all three stages of lactation but not in the suckling pups. The hepatic peroxisomal enzyme activities (PCoA and CAT) were increased by 5- to 8-fold in treated dams at all three stages of lactation. Two-fold increases in these enzyme activities were also observed in pups suckling the treated dams. Also hypo-lipidemia was observed in treated lactating rats at all three stages of lactation. Plasma cholesterol and triglyceride concentrations were decreased by 30-50%.

In a following experiment, the transfer of DEHP into milk of lactating rats was shown in groups of female Sprague-Dawley rats given 3 oral doses of 2,000 mg/kg bw/day of DEHP in corn oil by gavage on days 15-17 of lactation. Two hours after dosing on day 17, pups (10 per litter) were removed from the dams to allow milk to accumulate. Six hours after the last dose, the dams were killed and milk and mammary glands were collected. Two pups from each litter were killed 3-4 hours after the third dose. The rats were sacrificed 6 hours after the last dose. Increased activities of PCoA and CAT in dams and pups were observed also in this study. Mammary gland weights, both absolute and relative, were significantly reduced in treated rats. In treated rats, also total milk solids, lipid, and protein were increased relative to control rats, whereas milk lactose was significantly decreased. Milk collected 6 hours after the third dose contained 216 µg/ml DEHP and 25 µg/ml MEHP. In contrast, plasma contained virtually no DEHP (< 0.5 µg/ml) but

substantial amounts of MEHP (76 µg/ml) resulting in a high milk/plasma ratio for DEHP and a low milk/plasma ratio for MEHP. DEHP and MEHP were not detected in the plasma of the pups. After addition of (^{14}C)DEHP to milk *in vitro*, most of the radioactivity was associated with the fat globule layer.

In a study comparable to a guideline study, groups of 10 male Sprague-Dawley rats were given 0, 10, 100, 1,000 or 2,000 mg/kg bw/day of DEHP (> 99% pure) in corn oil by gavage for 5 days beginning at an age of 6 (1-week-old), 14-16 (2-week-old), 21 (3-week-old), 42 (6-week-old), or 86 (12-week-old) days (Dostal et al., 1987b) (see also Section 4.1.2.6.3). The control group was given the vehicle. After two doses of 2,000 mg/kg bw/day virtually all pups in the three youngest age groups died whereas 6- and 12-week-old rats showed significantly decreased body weights with no fatalities. Five daily doses of 1,000 mg/kg bw/day caused significant decreases in body weight gain in 1-, 2-, and 3-week-old rats. Absolute and relative liver weights were significantly increased at 100 mg/kg bw/day in all age-groups (except for 1-week-old rats) and in all age groups at higher dose levels. Absolute kidney weight was reduced in some cases whereas relative kidney weight was increased at doses of 1,000 mg/kg bw/day or more in 3-week-old rats or older rats. Morphological examinations revealed increased peroxisome proliferation in neonatal as well as adult rats. The activities of PCoA and CAT were increased in a dose-dependent manner in all age groups. The activities of these enzymes were similar in control rats of all ages. Plasma cholesterol concentrations were higher in suckling control rats (1- and 2-week-old) than in weanling (3-week-old) and adult controls. In DEHP-treated rats, plasma cholesterol concentrations were significantly reduced in weanling and adult rats given doses of 1,000 mg/kg bw/day or more. In suckling rats plasma cholesterol levels were increased at 1,000 mg/kg/ bw/day. Plasma triglyceride levels in the control group were similar at all ages whereas significant decreases in plasma triglycerides were observed in weanling and adult rats; in suckling rats only small decreases (not significant) occurred.

Tandon et al. (1990) performed a study on testis development in male albino rats after exposure during the nursing period. The aim was to study the effect of DEHP exposure through mother's milk on the enzymes in the testes considered to be markers of the testicular function in rats. Groups of four female rats were given vehicle (ground nut oil) or 2,000 mg/kg bw/day of DEHP (purity not specified) orally for 21 days from parturition. The pups were sacrificed at the age of 31, 61 and 91 days and and four animals from each group were used for histopathological studies of testes (fixed in formalin and embedded in paraffin), epididymis, prostate, and seminal vesicles. There were no signs of overt toxicity, significantly increased lethality or significant body weight change in the mothers. The light microscopic examination of the testes revealed no significant difference between treated and control animals. The offspring of DEHP-treated mothers showed, however, a significant increase in the activity of gamma-glutamyl transpeptidase, lactate dehydrogenase and beta-glucuronidase, and a significant decrease in the activity of acid phosphatase and sorbitol dehydrogenase at 31 and 61 of age compared to controls. No effect on these enzymes was seen in 91 days old rats. The authors conclude that exposure to DEHP during early life through mother's milk causes biochemical alterations which may affect the functional development of the testis.

Intravenous

Rats

In a 3-day-old neonatal rat model used to assess DEHP toxicity following intravenous administration, neonates (12 rats per group, 2 to 4 days old) were injected 30.8, 91.7 or 164.8 mg/kg bw of DEHP (purity not specified) in 4% bovine serum albumin (BSA) solution for

18 consecutive days (Greener et al., 1987). Control neonates were injected a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1 to 3 hours later. After sacrifice, a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum, and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to 21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were, however, detected in the tissues.

Embryo-culture systems

A 3-day-old neonatal rat model was used to assess DEHP toxicity following intravenous administration (Greener et al., 1987). Neonates (12 rats per group, 2 to 4 days old) were injected 30.8, 91.7 or 164.8 mg/kg bw of DEHP (purity not specified) in 4% bovine serum albumin (BSA) solution for 18 consecutive days. Control neonates were injected a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1 to 3 hours later. After sacrifice, a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum, and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to 21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were detected in the tissues with the exception of local lesions at the injection site (subacute dermatitis), also noted in half of the BSA and saline control rats.

Effects of DEHP on the chick embryo have been reported (Bower et al., 1970; Woodward, 1988). The results suggest that DEHP is capable of causing damage to the central nervous system of the developing chick embryo. The studies also demonstrated that phthalates have some potential for embryotoxic and teratogenic effects.

4.1.2.10.6 Metabolites of DEHP

A contribution to the developmental toxicity of DEHP might be the developmental and teratogenic effect of the metabolites MEHP or 2-ethylhexanol (2-EH). Therefore, available data on MEHP and 2-EH are included in this report.

Oral

Rats

Ten Female Wistar rats per dose group were daily administered 2-Ethylhexanol (2-EH) (0, 130, 650 and 1,300 mg/kg day in bidistilled water containing 0.005% Cremophor EL; > 99.5% pure) by gavage during gestation day 6 through 15 (BASF, 1991). The study was performed according to guidelines and GLP. On day 20 post coitum all surviving animals were sacrified and assessed by gross pathology. The foetuses were dissected from the uterus, sexed, weighed and further investigated for any external, soft tissue and/or skeletal findings. No adverse substance-related effects on dams of foetuses were observed in the 130 mg/kg dose group. In the 650 mg/kg dose group maternal (2 dams with piloerection) and embryo/fetotoxic effects (slightly reduced mean fetal body weights and increased frequency of foetuses with skeletal variations and retardations)

were found. In the 1,300 mg/kg dose group maternal toxic effects included markedly reduced food consumption during the whole treatment period, distinctly reduced mean body weights, reduced body weight, markedly reduced corrected body weight gain. Six animals were found dead on gestation days 9, 10 and 13; also severe clinical sypmtoms like abdominal or lateral position, unsteady gait and apathy, light brown-gray discoloration of the liver in the animals with intercurrent death; lung oedema and emphysema in a few animals, and haemometra in one dam which showed vaginal haemorrage before death, and distinctly reduced mean uterus weight. Embryo-/foetotoxic effects included increased number of resorptions and consequently markedly increased postimplantation loss, markedly reduced mean foetal body weights, increased incidence of foetuses with dilated renal pelvis and/or hydroureter, and a higher number of foetuses with skeletal malformations, variations, and retardations. The NOAEL for maternal and developmental toxicity was 130 mg/kg bw/day.

The effects of a single oral dose of 2-EH to pregnant rats were studied by Ritter et al. (1987). The doses of 0, 6.25 or 12.5 mmol/kg 2-EH (0, 833 or 1,666 mg/kg bw; purity not specified) were administered by gavage on gestation day 12 in Wistar rats. There is no information on GLP.

The group given 833 mg/kg showed a slight increase of 2% in malformed foetuses relative to the controls (0%). The other parameters (implantation index, mean foetal weight, number of dead and resorbed foetuses) were unaffected. Simultaneous intraperitoneal administration of 150 mg caffeine/kg potentiated this effect (increase in malformed foetuses to 21.2%). Even after a dose of 1,666 mg/kg, the implantation index and percentage of dead and resorbed foetuses were unchanged, although the mean foetal body weight at 3.5 g was reduced relative to the controls (4.1 g). 22.2 % of the surviving foetuses showed malformations (controls 0%). These included hydronephrosis (7.8%), tail anomalies (4.9%), anomalies of the extremities (9.7%), and "others" (1%). The LOAEL for maternal toxicity and the LOAEL for developmental toxicity is 833 mg/kg bw, the lowest dose tested.

Mice

In a study undertaken by Yagi et al. (1980) to determine the foetotoxicity of DEHP in mice (see Section 4.1.2.10.4) also the effects of MEHP were included. MEHP (dissolved in olive oil) was given to 2-8 females in doses of 0, 0.1, 0.5 or 1 ml/kg (corresponding to 1/15, 5/15 or 10/15 of the oral LD_{50}) on day 7, 8 or 9 of gestation. The administration of MEHP on day 7 or 8 of gestation resulted in high embryolethality at doses from 0.1 ml/kg. Oral administration of 0.5 or 1.0 ml/kg of MEHP on day 8 was also found to induce gross and skeletal abnormalities similar to those induced by DEHP. There is no information on maternal toxicity.

Tomita et al. (1986) investigated whether the embryotoxic/foetotoxic effects of DEHP are related to the metabolic formation of MEHP in the maternal body and/or in foetuses. Five to 7 ddY-SLC (SPF) mice were given a single oral dose of 0.1, 0.5 or 1.0 ml/kg on day 7, 8 or 9 of gestation.The MEHP induced lethality and malformation was dose- and time-dependent. The highest incidence of foetal death (less than 19% of live foetuses) was observed among mice given a dosage of 1 ml MEHP on day 7 of gestation. When MEHP was given on day 8 of gestation all live foetuses were malformed. Experiment with ^{14}C-DEHP showed the presence of both DEHP and MEHP in foetuses. The highest concentrations were observed in the pancreas. The concentration of DEHP increased with time. The level of MEHP in foetal tissue was about 1% that of DEHP. The concentration of both DEHP and MEHP in tissues varied greatly depending on the day of DEHP administration and were highest when administered on day 8 of gestation. According to the authors, the presence of MEHP in foetuses may be due to hydrolysis

of DEHP to MEHP in the maternal body followed by the transfer of MEHP across the placenta or a direct hydrolysis of DEHP to MEHP in the foetuses. From further experiments with tissue homogenates and foetuses from pregnant mice it could be assumed that MEHP found in the mouse foetuses is of maternal origin. Information on maternal toxicity is not included in the report.

In a study reported by Price et al. (1991a, b) and Tyl et al. (1991) female CD-1 mice were given 2-EH (0, 17, 59, or 191 mg/kg/day; > 99% pure) microencapsulated in the diet during gestation days 0 to 17. The study was performed according to the principles of GLP. No dams died, delivered early or were removed from the study. Pregnancy rate was high and equivalent across all groups. There was no treatment-related maternal toxicity observed in this study. There were no effects of exposure to dietary 2-EH on any of the gestational parameters. The number of corpora lutea, uterine implantation sites, pre- and postimplantation loss, sex ratio and live fetal body weight per litter were all equivalent across all groups. There were also no treatment-related changes in the incidence of individual, external, visceral, skeletal or total malformations or variations. In conclusion, there were no maternal or developmental toxic effects of 2-EH dietary exposure throughout gestation at any concentration tested. The NOAEL for maternal toxicity was identified to > 191 mg/kg bw/day and the NOAEL for developmental toxicity to > 191 mg/kg bw/day.

The principles of the Chernoff-Kavlok teratogenicity screening test (1982) were used in a study with female CD-1 mice administered 2-EH (0 or 1 525 mg/kg; purity not identified) in corn oil by gavage daily during gestation day 7 through 14 (Hardin et al., 1987). The study was performed according to the principles of GLP. The observation period lasted until day 3 post partum. The results of this study regarding the influence of 2-EH on reproduction should be taken with care since the dose applied resulted in the death of more than 30 % of treated dams. Therefore, the observed effects on the offspring should be attributed to the extensive maternal toxicity and are very unlikely to be primary effects. The authors state that the results of this assay should not be used to label a chemical as teratogenic or nonteratogenic but to establish priorities for conventional testing. This screening test was conducted with one group of 50 pregnant CD-1 mice. The dose of 1,525 mg 2-EH/kg/day was determined previously as the minimal effective dose for adult female mice. In 17 animals, test substance related mortality was observed by the end of the treatment period. Another animal died because of a dosing error. Clinical observations in dams included languidity, ataxia, coldness to touch, wet stains, oily coat, and dark red discharge from the anus of one animal. Decreases in body weights and the reproductive index were observed in treated animals compared to controls. Decreases were also observed in the following parameters when compared to controls: mean number of live pups per litter, litter weight, pup weight on days 1 and 3, percent change in pup weight from day 1 to day 3, mean pup viability per litter from day 1 to 3, and percent of live pups per litter on day 1 post partum. The mean number and percent of dead pups in the treatment group was reported to be greater than in the control group.

MEHP (0, 35, 73, 134 or 269 mg/kg/bw) administered to CD-1 mice (25-27 animals per group) on gestational days 0 to 17 was shown to cause developmental toxicity and malformations at doses from 35 mg/kg bw/day (NTP, 1991). A developmental LOAEL of 35 mg/kg bw may be derived from this study.

Inhalation

Rats

Female Sprague-Dawley rats were exposed to 0 or 850 mg/m^3 2-EH (the highest concentration that could be generated as a vapour; > 99% pure) during gestation days 1 to 19 (Nelson et al., 1989). The study was performed according to the principles of GLP. Dams were sacrificed on day 20. Reduced maternal feed intake but no malformations were demonstrated at the dose level administered.

Dermal

Rats

Administration of 2-EH (> 99.7%) by occluded cutaneous application 6 hours per day to pregnant Fischer 344 rats (25 animals per dose group) during organogenesis (gestation day 6 through 15) at 0, 0.3, 1.0 or 3.0 ml/kg/day (0, 252, 840 and 2,520 mg/kg/day) resulted in maternal toxicity at 1.0 and 3.0 ml/kg/day (Tyl et al., 1992). The study was performed according to US EPA Health Effect Guidelines and GLP. Clinical signs of toxicity were observed at the dosing site for the two highest dose groups. At 3.0 ml/kg/day also reduced weight gain in the treatment period was observed. No developmental toxicity and no treatment-related increased incidence of malformations were noted at any dosage employed. The NOAEL for maternal toxicity was 0.3 ml/kg/day (252 mg/kg bw/day) and for developmental toxicity 3.0 ml/kg/day (> 2,520 mg/kg bw/day).

Intravenous

Rabbits

In a study conducted in rabbits, MEHP was given intravenously in saline (1.14, 5.69 or 11.38 mg/kg bw) to groups of 11 females on day 6 to 17 of gestation (Thomas et al., 1979, cited by Woodward, 1988; Thomas et al., 1986). Maternal toxicity was reported as 2 and 3 of the rabbits died in the mid- and high-dose groups, respectively, but one control animal given only saline also died. A significant effect on foetal development was noted at the highest dose level. A high incidence of resorptions (21%) and post-implantation losses occurred in the high-dose group, whereas only a low incidence was seen in control animals (5-6%).

4.1.2.10.7 Studies in humans

No human reproductive toxicity data are available.

4.1.2.10.8 Studies investigating endocrine activity

In the last few years, a hypothesis has been put forward that many man-made substances, including phthalates, may interfere with the normal functioning of the human endocrine system and cause disorders of the human reproductive and endocrine systems, particularly with regard to reproductive cycle and reproductive function, breast and testicular cancer, learning and behavioural problems, and immune system deficiencies (MST, 1995; Kavlock et al., 1996; Barton and Andersen, 1997).

The endocrine system consists of complex feedback pathways involving the brain and the endocrine organs. The normal functions of all organ systems are regulated by endocrine factors, and small disturbances in endocrine function, especially during certain stages of the life cycle such as development, pregnancy, and lactation, can lead to profound and lasting effects.

One group of hormones, the oestrogens, have received special attention as they play an important role in many developmental and physiological responses. Oestrogens are involved in sexual development and exposure to high oestrogen concentrations during critical periods of development may lead to teratogenic and carcinogenic lesions in the reproductive tracts of humans. Furthermore, oestrogens may be implicated in the initiation and progression of breast, ovarian, endometrial, and prostate cancers (Gaido et al., 1997).

Endocrine-active substances (disrupters) are very often considered as being synonymous with substances that interfere with the function of the oestrogens. However, substances which may interfere with androgens or with other components of the endocrine system such as the thyroid and pituitary glands are also covered by the definition of endocrine-active substances.

The endocrine activity of DEHP has been investigated both *in vivo* and *in vitro*. The *in vitro* models include a recombinant yeast screen, a whole ovary culture assay, a trout hepatocyte vitellogenin assay, oestrogen sensitive MCF-7 and ZR-75 cells, and Sertoli cell cultures. None of the studies have, however, been validated and internationally accepted as test methods. Of the non-regulatory *in vitro* test models, the MCF-7 cell lines, the trout vitellogenin assay for oestrogenic activity, and the yeast cell assay for oestrogenicity and androgenicity have been used for several years and are included in the proposed test models for further development with a view to possible adoption as OECD test designs.

4.1.2.10.9 Studies in animals

Regularly cycling Sprague-Dawley rats (6-9 animals per group) were dosed daily with 2,000 mg/kg bw of DEHP by gavage in corn oil for 1-12 days (Davis et al., 1994a). A control group was given the vehicle. Ovarian morphology and levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and progesterone were determined. Exposure to DEHP resulted in prolonged oestrus cycles and suppressed or delayed ovulation by the first pro-oestrous/oestrus after the meta-oestrous initiated dosing. Histopathological examination of the ovaries demonstrated that 7 out of 10 DEHP-exposed rats had not ovulated by vaginal oestrus in contrast to 13 out of 13 control rats. Pre-ovulatory follicles were quantitatively smaller in DEHP-exposed rats than in controls due to smaller granulosa cells. Serum oestradiol levels were significantly decreased and serum FSH levels were significantly increased. No significant differences were detected in serum progesterone levels. Results for LH were equivocal. According to the authors, exposure to DEHP results in hypo-oestrogenic anovulatory cycles in adult female rats.

Agarwal et al. (1986 a,b) administered DEHP (> 99% pure) to groups of sexually mature 24 male F344 rats (age about 15 weeks) in the diet at 0, 320, 1,250, 5,000 or 20,000 ppm (equivalent to doses of 0, 18, 69, 284 and 1,156 mg/kg bw) per day for 60 days (see also Section 4.1.2.9.1). There was a trend towards decreased testosterone and increased LH and FSH levels in serum at 5,000 and 20,000 ppm. The authors suggested that a restricted release of testosterone into the blood would be expected to increase FSH and LH concentrations by a compensatory mechanism, the observations therefore suggest that exposure to DEHP may alter circulating androgen release at the level of testis.

The effects of DEHP on 17β-hydroxysteroid dehydrogenase (HSD) activity in rat testes were studied by Srivastava and Srivastava (1991). This enzyme is considered to be a marker of Leydig cell function and a key enzyme in steroidogenesis. Male Wistar rats were given 0, 250, 500, 1,000 or 2,000 mg/kg bw/day of DEHP (highest purity commercially available) by gavage in groundnut oil for 15 days. The activity of testicular HSD was significantly decreased from 1,000 mg/kg bw/day. These results suggest, according to the authors, that DEHP may adversely affect steroidogenesis in the testes and, subsequently, Leydig cells may be involved in the testicular toxicity of DEHP.

In another study, male Fischer 344 rats (18 animals per group) were fed a diet containing 0 or 1.2% DEHP (purity not specified) (Eagon et al., 1994). After 4, 8, and 16 weeks of treatment, 6 animals from both treatment and control groups were sacrificed. Oestrogen receptor (ER) activity was significantly reduced in cytosolic and nuclear fractions from livers of rats treated for 8 and 16 weeks. Serum oestradiol levels were significantly elevated at all exposure times. Hepatic microsomal oestrogen 2-hydroxylase was significantly reduced after 4 and 8 weeks, and slightly, but not significantly, after 16 weeks. Male oestrogen binding protein and oestrogen receptor mRNA were significantly decreased after 8 weeks. According to the authors, the observed changes in hepatic oestrogen metabolism together with the induced hyperplasia could play a crucial role in the hepatocellular carcinogenesis induced by DEHP in rats.

In a study of the effects of DEHP on the thyroid, male Wistar rats (18 rats per group) were fed diets containing 0 or 1% of DEHP (purity not specified) (Hinton et al., 1986) (see also Section 4.1.2.6.3). Six rats from the treatment group and six controls were sacrificed after 3, 10 or 21 days of feeding. A significant decrease in serum levels of thyroxin (T_4) was observed whereas the levels of serum triiodothyronine (T_3) were unaffected (slight, not significant alterations). Electron microscopic examination of the thyroids of treated rats showed marked ultrastructural changes (increase in the number and size of lysosomes, enlargement of the Golgi apparatus, and damage of mitochondria) indicative of hyperactivity of the thyroid gland.

In a study investigating the effect of DEHP on serum levels of thyroid hormones, intact male Sprague-Dawley rats and thyroidectomised male rats with parathyroid replants (5-6 animals per group) were given 1,200 mg/kg bw/day of DEHP by gavage in corn oil for 7 days (Badr, 1992). One group of intact and one group of thyroidectomised rats were subcutaneously administered T_4 in saline (400 and 200 µg/kg bw/day, respectively). A vehicle group was included for each treatment group. Total concentration of T_4 and T_3 as well as free T_3 (fT_3) in serum was measured by solid phase radioimmuassay. In intact rats, DEHP did not alter serum levels of the thyroid hormones when compared to controls. When thyroidectomised rats were supplemented with T_4, the serum hormone levels were elevated when compared to intact controls. DEHP lowered these levels significantly when given to either intact or thyroidectomized rats supplemented with T_4. Hormone levels were below the detection limit in thyroidectomized rats given corn oil or DEHP. The authors suggested that DEHP enhances the metabolism and/or excretion of thyroid hormones.

In a male fertility study, Gray and Gangolli (1986) studied the effects of MEHP on Sertoli cell function by measuring the secretion of seminiferous tubule fluid and androgen binding protein (see Section 4.1.2.10.1). A single dose of 1,000 mg/kg bw. MEHP reduced protein production to around 50% of the concurrent control group and to 25% after three repeated doses.

Zacharewski et al. (1998) investigated *in vitro* the estrogenic activity of eight phthalate esters using an estrogen receptor competitive ligand-binding assay and mammalian (human breast cancer MCF-7 and HeLa cells) and yeast-based gene expression assays. In addition the effect on uterine weight and vaginal cell cornification *in vivo* using ovariectomized immature and mature

(Sprague -Dawley) rats, respectively, was examined. No significant responses were observed in any of the *in vitro* or *in vivo* assays with DEHP.

A recently performed 2-generation range finding study in Wistar rats (cited in more detail in Section 4.1.2.10.1 and 4.1.2.10.4) indicates a specific disturbance of the male sexual differentiation (Schilling et al., 1999). The incidence of areolas/nipple anlagen was significantly increased in male high-dose F1 pups (83.9%) at 9,000 ppm and the sexual maturation (based on preputial separation in male pups and vaginal opening in female pups) was significantly retarded. A dose related loss of spermatocytes was found at 3,000 and 9,000 ppm (2/10 and 7/9, respectiveley). In F1 parental animals the absolute and relative liver and testicular weights and the absolute epididymidal weight were significantly decreased at 9,000 ppm. The testes and the epididymides were reduced in size in three out of six animals at 9,000 ppm. There was a dose-related decrease of the prostate weight from 1,000 ppm. The substance related effect on the gonads was confirmed by histopathological findings (see Section 4.1.2.10.1). Female interior organs were unaffected. Also in male F2 pups the anogenital distance and anogenital index were reduced.

The study results indicate the specific sensitivity of male pups when exposed to DEHP during early development and a specific action on male genital organs such as the testicle, the epididymis, and the prostate including decreased reproductive organ weights, germ cell loss in the testis of the male offspring, nipple anlagen, and reduced anogenital distance.

Based on these effects on the F1 and F2 generation males it is plausible that DEHP interferes with male specific differentiation factors among which the action of androgens is the most important.

Studies in rats exposed to di-*n*-butyl phthalate (DBP) have shown similar findings (Mylchreest and Foster, 1998). Administration of DBP by gavage during gestation and lactation produced decreased anogenital distance, absent or underdeveloped epididymis and seminal vesicles, hypospadia, decreased reproductive organ weights, and widespread germ cell loss in the testis in the male offspring of CD rats. The findings indicated that DBP was unlikely to be an oestrogen as there was no effect on oestrogen-depending events such as vaginal opening, age at first oestrus, and oestrus cyclicity in the female offspring. The responses observed were more similar to those reported for the anti-androgen flutamide, although some responses were not. The major targets of DBP in the differentiating male reproductive tract were androgen-dependent.

Mylchreest et al. (1998) hypothesized that DBP is antiandrogenic due to the reproductive tract malformations in several androgen dependent tissues in male offspring. Based on the high incidence of testicular and epididymal lesions in DBP-treated male offspring, they proposed that DBP alters reproductive development by a different mechanism of action than the known androgen receptor antagonists flutamide or Vinclozolin. This was confirmed by Gray et al. (1999), who found that toxic substances can alter sexual differentiation in an antiandrogenic manner via several distinct mechanisms. They investigated the reproductive effects of ten known or suspected anti-androgens, including flutamide, Vinclozolin, DBP, and DEHP.

Eight pregnant Sprague Dawley dams were administered DEHP (750 mg/kg bw/day; > 99% pure) in corn oil by gavage from gestation day 14 to day 3 of lactation Gray et al. (1999). The male offspring (killed at about 5 months of age) was examined for abnormalities such as retained nipples, cleft phallus, vaginal pouch, and hypospadias. The animals were also examined internally (ectopic or atrophic testes, agenesis of the gubernaculum, epididymides, sex accessory glands, and ventral prostate, epididymal granulomas, hydronephrosis, and enlarged bladder with stones). Weights measured included body, pituitary, adrenal, kidney, liver, ventral prostate,

seminal vesicle (with coagulating gland and fluid), testis, and epididymis. Gonads and sex accessory tissues were examined microscopically.

DEHP was considerably more toxic than was DBP to the reproductive system of the male offspring. The gestational and lactational exposure induced a statistically significantly increased incidence of both reproductive and non reproductive malformations including decreased anogenital distance, areolas (88%), hypospadias (67%), vaginal pouch (45%), ventral prostate agenesis (14%), testicular and epididymal atrophy or agenesis (90%), and retained nipples. In addition, several 8-day old pups displayed haemorrhagic testes by gross examination. In the adult offspring (5 months of age) the weight of the gonads, the accessory sex organs, and the muscle Levator ani-bulbocavernosus were significantly decreased.

Gray and coworkers found that the chemicals investigated could be clustered into three or four separate groups, based on the resulting profiles of reproductive effects. DBP and DEHP induced a higher incidence of testicular and epididymal abnormalities, including atrophy and agenesis, which is not generally found with flutamide or Vinclozolin even at high dose levels.

4.1.2.10.10 *In vitro* studies

Whole ovary cultures from cycling Sprague-Dawley rats fed 1,500 mg/kg bw/day of DEHP (purity not specified; in corn oil) by gavage for 10 days were used to evaluate if DEHP altered steroidogenic profiles (Berman and Laskey, 1993). Ovaries were removed and cultured for one hour. Steroidogenic profiles of progesterone, testosterone, and oestradiol release into the medium were measured using radioimmunoassay techniques. Dioestrous ovaries produced more oestradiol after DEHP administration and oestrus ovaries significantly less oestradiol; proestrous ovary production was not significantly changed. Testosterone production was significantly increased only in dioestrous. DEHP had no significant impact on progesterone production or serum levels of progesterone and oestradiol in treated rats.

Jobling et al. (1995) used different *in vitro* systems to study the oestrogenic activity of a variety of chemicals, including DEHP. The initial screening for oestrogenicity was carried out to measure the direct binding of chemicals to the fish oestrogen receptor. In a cytosolic extract from rainbow trout liver, 1mM DEHP (purity not specified) reduced the binding of tritiated 17β-oestradiol to the receptor to about 75% of the control value whereas a concentration of 0.001 mM DEHP had no effect.

The chemicals were also tested in two oestrogen-responsive human breast cancer cell lines, ZR-75 and MCF-7. When tested for its mitogenic effects on cell growth in ZR-75 cells, DEHP did not stimulate cell growth at a concentration of 0.01 mM. Cells were cultured for 10 days and counted on days 0, 3, 6, 8 and 10. All experiments were carried out in duplicate and repeated twice. When tested for its ability to stimulate the transcriptional activity of the oestrogen receptor directly in MCF-7 cells, DEHP did not stimulate transcription to any appreciable degree until concentrations in excess of 0.1 mM were reached. At this concentration, the response was less than 15% of the maximum response obtained with oestradiol.

Two other phthalates (DBP and BBP) were identified as oestrogenic substances in these *in vitro* systems when tested at concentrations between 0.001 and 0.1 mM.

A large number of phthalate esters, including DEHP, were screened for oestrogenic activity using a recombinant yeast screen at concentrations ranging from 10^{-3} M to $5 \cdot 10^{-7}$ M (Harris et al., 1997). Two cells lines, MCF-7 and ZR-75 were used for testing for mitogenic effects. In the yeast screen, some phthalates, such as BBP, (DBP) and (DIBP), were found to possess a weak

oestrogenic activity by generating a dose-dependent increase of β-galaktosidase production, DEHP did not. The results from the MCF-7 and ZR-75 assays were comparable to those obtained from the yeast screen. The authors suggested that the phthalates were not fully solubilized in the water-based medium why it may be plausible that some of the phthalates tested could actually be more potent than they appeared to be.

Four phthalate diesters, including DEHP, were investigated *in vivo* for effects on Leydig cell structure and function (Jones et al., 1993) (see also Section 4.1.2.10.1). The corresponding monoesters were investigated *in vitro*. The study was performed due to earlier study results indicating that communication and control exists between Leydig and Sertoli cells which appear to be of a paracrine nature. In the *in vivo* study, testicular tissues were studied by light and electron microscopy after glutaraldehyde perfusion fixation, Taab embedding and toluidine blue staining (a highly reliable technique in preparing testis tissue for identifying testicular toxicity). The *in vitro* study was performed with primary cultures of Leydig cells incubated with MEHP. The results showed that MEHP and DEHP exerted a direct effect on Leydig cell structure and function (as determined by testosterone output) with correlation of the *in vitro* and *in vivo* effects of, respectively. The changes observed *in vivo* were present in all animals in each group. In Leydig cell cytoplasmatic ultrastructure, several subtle but highly significant alterations were produced. DEHP administration also resulted in slight rarefaction or vacuolation of a few Sertoli cells in seminiferous tubules. In the *in vitro* study, MEHP, produced marked effects on structure and function in Leydig cells, including decreased LH-stimulated secretion of testosterone. The results indicate that DEHP exerts a direct effect on Leydig cell structure and function and that DEHP and MEHP produce similar changes both *in vivo* and *in vitro* both in Leydig and Sertoli cells. The authors concluded that a malfunction of Leydig cells likely affects the physiology of adjacent Sertoli cells.

The effects of DEHP and MEHP on rat Sertoli cells *in vitro* was also studied by Grasso et al. (1993) (see also Section 4.1.2.10.1). MEHP was found to specifically reduce the ability of FSH to stimulate cyclic adenosine monophosphate (cAMP) accumulation in cultured Sertoli cells from rats, 13-82 days of age. This inhibition by MEHP of FSH-stimulated cAMP accumulation had a lag period of 6 hours and reached a maximal inhibition of 40-60% after 24 hours. Preincubation of Sertoli cells for 24 hours with 100 μM DEHP had no effect on FSH binding. The authors concluded that the ability of certain phthalate esters to reduce FSH binding to Sertoli cell membranes is likely to be a part of the mechanism responsible for their testicular toxicity.

4.1.2.10.11 Metabolites of DEHP

The effect of MEHP on rat granulosa cell function has been studied by Treinen and Heindel (1992). It was shown that MEHP inhibited FSH-stimulated cAMP (cyclic adenosine monophosphate) accumulation. The effect was specific for FSH as MEHP had no effect on the ability of forskolin or isoproterenol to stimulate cAMP accumulation. MEHP also caused a dose-dependent decrease in FSH-stimulated progesterone production, a cAMP-dependent process.

In another similar assay, rat granulosa cells were obtained from female Fisher 344 rats and cultured in the presence of various concentrations of MEHP (0 to 400 μM) (Davis et al., 1994b). The granulosa cells were stimulated with FSH or a cAMP analogue (8-bromo cyclic adenosine monophosphate). Oestradiol production was measured by radioimmunoassay. MEHP suppressed oestradiol in a concentration-dependent manner whether granulosa cells were stimulated by FSH or the cAMP analogue indicating that MEHP suppressed oestradiol independently of its suppression of the FSH-cAMP pathway and, thus, suppressed aromatase conversion of

testosterone to oestradiol. The aromatase activity was determined by measuring granulosa cell oestradiol production at various concentrations of testosterone. MEHP (100 μM) significantly decreased the maximal activity of aromatase.

4.1.2.10.12 Summary of toxicity for reproduction

Available data demonstrate that exposure to DEHP affects both fertility and reproduction in rodents of both sexes and also produces developmental effects in offspring. In males, DEHP induces severe testicular effects, including testicular atrophy. Developing male rats have been found to be more sensitive to DEHP-induced testicular toxicity than sexually mature animals (Gray and Butterworth, 1980; Sjöberg et al., 1985c, 1986b; Wolfe et al., 2003). The onset of the lesion in young animals is also more rapid. Irreversible effects occur in rats exposed prenatally and during suckling (Arcadi et al., 1998).

MEHP is believed to be the active metabolite of DEHP affecting testes and reproductive functions both *in vivo* and *in vitro*. The possible role of other metabolites is, however, not fully elucidated.

Testicular effects have been observed in several repeated dose toxicity studies in rats, mice, ferrets, (Gray et al., 1977; NTP, 1982; ICI, 1982b; CMA, 1984b,c; Ganning et al., 1990; Eastman Kodak, 1992a; Moore, 1996; Poon et al., 1997; Lamb et al., 1987; NTP, 1982; Moore, 1997; Lake et al., 1976; 1986, Gray et al., 1982; Schilling et al., 2001; Wolfe et al, 2003). In addition, minor effects were obsereved in hamster exposed to DEHP and more server effcets induced by MEHP (Gray et al., 1982). In the available studies marmosets were not sensitive to DEHP (Kurata et al., 1995; 1996; 1998). No studies on testicular effects in rabbits are available.

The NOAEL for testicular effects, as identified in a guideline three-generation reproductive toxicity study (Wolfe et al., 2003), is 4.8 mg/kg/day. A NOAEL of 3.7 mg/kg bw in rats was indicated based on a high incidence (7/9) of Sertoli cell vacuolation at the next higher dose level (500 ppm equivalent to 37.6 mg/kg body weight) in a 13-week guideline study (Poon et al., 1997). At the highest dose level (5,000 ppm equivalent to 375.2 mg/kg body weight) also a high incidence of atrophy of the seminiferous tubules with complete loss of spermatogenesis was found in addition to a higher incidence of cytoplasmic Sertoli cell vacuolation (9/10). The methodology used for the preparation of the testicular tissue (paraffin embedding, haematoxylin-eosin staining, and light microscopy) is generally adopted, but not as currently recommended in the guidelines. However, the progressive increase in vacuolation of Sertoli cells plus injury and loss to germinal epithelium and spermiogenesis in a treatment-related fashion is regarded as a strong evidence that the conclusions were not compromised by the methodology employed. Vacuolation has been found to be an early morphological sign of a testicular injury and the cardinal response seen with many of the Sertoli cell toxicants and also a marker of subsequent functional changes (inter alia Fawcett, 1975; Courtens and Plöen, 1999). The presence of multiple, small vacuoles in the basal Sertoli cell cytoplasm has been found to be a prominent feature of the early response to phthalate exposure in young rats (Creasy et al., 1983). However, as there remains some doubts as to the toxicological significance of the sertoli cell vacuolisation observed in the Poon study, a NOAEL of 4.8 mg/kg/day (100 ppm) is chosen from the Wolfe study (2003) for the risk characterisation, based on occurrence of small male reproductive organs (testis/epididymes/seminal vesicles) and minimal testis athrophy (exceeding those of the current controls as well as historical control groups) at 300 ppm and above.

Both *in vivo* and *in vitro* experiments have demonstrated that the Sertoli cell is one of the the main target of DEHP/metabolite-induced testicular toxicity producing subsequent germ cell

depletion (Poon et al., 1997; Arcadi et al., 1998; Li et al., 1998). Sertoli cells provide both physical support as well as secreting factors that are required for germ cell differentiation and survival and may also influence the signal transduction mechanism between these cells. Findings from an *in* vitro study have also shown that phthalate-induced changes in germ cell-Sertoli cell adhesion may occur during early postnatal development in rats. The Sertoli cells are also the principal testicular site for the action of FSH, a hormone which is essential for initiation and maintenance of spermatogesis. In pubertal animals FSH is more important than in adults due to the initiation of spermatogenesis. The relatively rapid onset of phthalate-induced testicular injury suggests a specific mechanism of action on Sertoli cells. The four distinct mechanistic hypotheses which have been proposed to explain testicular injury implicate zinc-dependent enzyme activity, hormonal status, metabolic interactions, and FSH-dependent pathways. Recent research results suggest that the FSH-stimulation of Sertoli cells is decreased by DEHP.

Study results have also shown that DEHP and MEHP may exert a direct effect on Leydig cell structure and function as determined by testosterone output and also that DEHP and MEHP produce similar changes both *in vivo* and *in vitro* both in Leydig cells and in Sertoli cells (Jones et al., 1993). It is plausible that malfunction of Leydig cells affects the physiology of adjacent Sertoli cells. Findings also indicate that different phthalates may exert changes that are unique to one or common to both cell types.

Developing and prepubertal rats have been found to be much more sensitive to exposure to DEHP than adults (Gray and Butterworth, 1980; Sjöberg et al., 1985c; 1986b, Arcadi et al., 1998; Wolfe et al., 2003). The younger animals respond to a much lower dose or produce a more serious lesion with a comparable dose on a mg/kg/day basis. In some instances, the onset for the production of the lesion is also more rapid. Exposure of rats prenatally and during suckling has produced irreversible effects at dose levels inducing only minimal effects in adult animals at the same exposure levels (Arcadi et al., 1998; Wolfe et al., 2003). In the 90-day study conducted by Poon et al. (1997), rats were dosed at 32-37 days of age and reach sexual maturity at approximately 70 days (Charles River, 2000). Since the rats were only immature for part of the dosing (33-38 of 90 days) and the study did not discern an age-dependent effect, the results of this study are considered relevant for both young and adult males. Furthermore, humans are exposed to DEHP for their whole lifetime, i.e. prenatally to death, via the environment, consumer products and medical devices. In addition, occupational exposure may occur.

DEHP has been observed to decrease the levels of zinc in the testes and testosterone in rodents (e.g. Oishi and Hiraga, 1980; Oishi, 1986; Agarwal et al. 1986a,b). Zinc-deficient and low protein diets have been shown to enhance the susceptibility to the gonadotoxic effect in adult males (Agarwal et al., 1986a). Co-administration of zinc did not, however, prevent the atrophy (Oishi and Hiraga, 1983): DEHP may interference with gastrointestinal absorption of zinc rahter than causing a directc effect on the testes. Co-administration of testosterone or the vitamin B_{12} derivative adenosylcobalamin with DEHP to male rats appears to prevent testicular injury (Parmar et al., 1987; Oishi, 1994). A low protein diet has been shown to enhance the susceptibility to the gonadotoxic effect (Tandon et al., 1992)

Based on the available data, which varies in both the study designs and number of animals included, testicular effects have been demonstrated in both male rodents and non-rodents: rat (NOAEL = 3.7 and 4.8 mg/kg bw/day) mouse (NOAEL = 98.5 mg/kg bw/day), and the ferret (LOAEL = 1,200 mg/kg/day) (Poon et al., 1997, Moore, 1997; Lake et al., 1976). In addition, minor effects were obsereved in hamster exposed to DEHP and more server effcets induced by MEHP (Gray et al., 1982). In the available studies with marmosets testicular toxicity has not been observed after treatment with DEHP (Kurata et al., 1995; 1996; 1998). The reasons for the differences in study results has been suggested to concern toxicokinetic considerations and

altered zinc homeostatsis. Moreover, other factors such as animal age, study design, animal model selection have to also be considered. For instance, marmosets which are new-world monkeys vary in their metabolic pathways and capacities and are not as closely related to humans as are cynomolgus and Rhesus monkeys (old-world monkeys) Caldwell, (1979 a,b). In a recent publication, the use of the marmoset monkeys rather than neonatal macaques apes was recommended because Sertoli cell replication is negligible neonatally in the latter species (Sharpe et al., 2000). Experimentally, by modullating Sertoli cell replication with a gonadotropin-releasing hormone antagonist, the authors also compared marmosets and rat (Wistar). They showed that marmoset apes and neonatal rats are similar. However, perinatal rats, unlike infantile and adult marmosets, lack replication. Although Sertoli cell replication seems to be more similar in man and marmosets, and the efficiency of spermatogenesis is poor in marmosets as well as in humans, there is, however, no evidence to support that the results obtained in prepubertal rats are not relevant for man or that use of adult marmosets should be prefered. Other mechanism(s) and/or factors that cause the observed differences in the DEHP-induced testicular toxicity have not, however, been fully substantiated. Based on the available animal data it is not possible to definitely conclude the relevance of these differences in humans. However, in the limited toxicokinetic data in humans, MEHP, the testicular toxicant, is formed following exposure to DEHP. Therefore, DEHP-induced testicular effects observed in animal studies are considered relevant for humnas, and the NOAEL of 4.8 mg/kg bw/day (Wolfe et al., 2003) is selected for the risk characterisation of humans.

Effects on male fertility have been observed in mice and rats. In mice, DEHP adversely affects the number of fertile matings. In a continuous breeding study an oral NOAEL of 0.01% in the diet (20 mg/kg bw/day) was identified for fertility (Lamb et al., 1987). In rat, the oral NOAEL for body weight, testis, epididymis, and prostate weights and for endocrine and gonadal effects in male rats was considered to be 69 mg DEHP/kg bw/day in a 60 day study (Agarwal et al, 1986 a,b). In a complementary crossover mating trial, females given 0.3% DEHP were more seriously affected than males. None of the females were able to produce pups: the fertility index was 0 (0/16) for females and 20% (4/20) for males compared to 90% for the control group (18/20).

There are indications that oral dosing of DEHP causes hypo-oestrogenic anovulatory and polycystic ovaries in adult female rats (Davies et al., 1994a, b). There also are indications that DEHP treatment alters the oestrus cycle and causes concentration changes of testosterone and oestradiol as shown in ovary cell cultures with cells obtained from cycling female rats administered DEHP *in vivo*. No NOAEL or LOAEL has, however, been established for these effects.

Effects on developmental toxicity have been observed in several studies. The rat has been shown to be the most sensitive species to DEHP-induced malformations. Irreversible testicular damage in the absence of obvious effects on the dams was shown in male pups exposed *in utero* and during suckling at very low dose levels (LOAEL = 3.5 mg/kg bw/day) (Arcadi et al., 1998). Their mothers were exposed to DEHP in drinking water at doses from about 3 mg/kg/day during pregnancy and lactation. However, there is some uncertainty with regard to the actual concentration of DEHP in the water. Alterations in kidneys tended to ameliorate with time; the testicular lesions did, however, not appear to reduce with growth. Histopathological changes were still observed at termination of the study, 8 weeks after delivery. The same levels of exposure did not produce similar effects in adult male rats. Effects on the male reproductive system, partly induced during the gestational period, were also observed in a three-generation study with a NOAEL of 4.8 mg/kg/day (Wolfe et al., 2003). In mice, DEHP is embryotoxic and teratogenic at oral dose levels below those producing observable evidence of toxicity to the dams:

In a continuous breeding study in mice, an oral NOAEL for maternal and developmental toxicity of 600 and 20 mg/kg bw/day were identified, respectively (Lamb et al., 1987). In a developmental toxicity study an oral NOAEL was identified as 44 mg/kg bw/day. The NOAEL for maternal toxicity was 91 mg/kg bw/day (NTIS, 1984; Tyl et al., 1988). In a dietary 2-generation study in mice, the maternal NOAEL was 0.05% DEHP (91 mg/kg bw/day) and the NOAEL for F1 offspring 0.025% (48 mg/kg bw/day) (NTIS 1988).

A few developmental toxicity studies have been performed in other species. These studies are, however, inconclusive. Only one developmental study is available concerning the effects of exposure to DEHP by inhalation (Merkle et al., 1988). However, this study is not considered reliable for risk characterisation. Because of uncertainties with regard to the actual dosing in the study by Arcadi et al. (1998), which has given the lowest effect level, the NOAEL of 4.8 mg/kg/day (Wolfe et al., 2003) is selected for risk characterisation of humans.

Animal data have also shown that DEHP and its metabolites can be transferred to pups via mothers milk in concentrations sufficient to cause toxicity (Parmar et al., 1985, Dostal et al., 1987a, Tandon et al., 1990).

Both *in vivo* and *in vitro* study results indicate that DEHP can interfere with the endocrine function and also influence the sexual differentiation (e.g. Gray et al., 1999 and Jones et al., 1993). Due to the effects on the Leydig cells as measured by a decreased testosterone output, it cannot be excluded that DEHP may exert an antiandrogen effect. The results of recently performed *in vivo* studies in rats exposed to DEHP or DBP support the hypothesis that exposure to phthalates may be provoked by an antiandrogen mechanism (Gray et al., 1999, Mylchrest and Foster, 1998). The present data in experimental animals are of concern for humans.

According to Council Directive 67/548/EEC, the categorization of substances toxic to reproduction may be classified under two main headings:

1) Effects on male or female fertility Data from well performed rat and mouse studies showing effects on fertility in males and females. Furthermore, DEHP has been shown to be a testicular toxicant in several species (rat, mice, ferret and hamster). Also young developing rats are more sensitive to the effects on the testes, after oral dosing of DEHP. These data are considered adequate to support the possibility that these effects can occur in humans. Hence, DEHP has been classified in Category 2, R 60.

2) Developmental toxicity. Well performed studies in rats and mice have shown developmental effects at dose levels not causing maternal toxicity. Hence, DEHP has been classified in Category 2, R 61.

3) Effects during lactation: It has been documented that DEHP is secreted into the milk of rats orally exposed to DEHP during the lactation period resulting in changes of the milk composition and also adverse effects in suckling pups (reduced body weight and induction of peroxisomal enzyme activities). DEHP has also been found in infant formulas and mothers milk.

Table 4.58 Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
Male reproductive studies			
Rat, Sprague-Dawley 17/males/group	3 generations via diet; 1.5, 100, 300, 1,000, 7,500 and 10,000 ppm (0.1, 0.5, 1.4, 4.8, 14, 46, 359 and 775 mg/kg/day	dose-dependent effets on numerous testis-related parameters. NOAEL for test.tox and dev. tox. 4.8 mg/kg/day, and 46 mg/kg/day for fertility	Wolfe et al, 2003
Rat, Wistar, 25 animals/group	0, 1,000, 3,000 or 9,000 ppm DEHP via the diet (corresponding to approximately 0, 113, 340, or 1,088 mg/kg/day)	3,000 ppm; a reduced testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring. Minimal focal tubular atrophy also occurred at 1,000 ppm (113 mg/kg and day), which thus constitutes a conservatively chosen LOAEL	Schilling et al., 2001
Rat, Wistar 10 males/group	4 weeks, *inhalation*, 0, 10, 50 or 1,000 mg/m^3	no effects on male fertiliy, no testicular toxicity NOAEL 1,000 mg/m^3	Klimisch et al. (1992)
Rat, F344 24 males/group	60 days, *diet* 0, 320, 1,250, 5,000 or 20,000 ppm (0, 18, 69, 284 or 1,156 mg/kg bw/day)	Dose-dependent ↓ in total body, testis, epididymis, and prostate weights from 5,000 ppm ↓mean litter size at 20,000 ppm correlated with degenerative testicular changes, ↓ testicular zinc content, epididymal sperm density and motiliy, ↑ number abnormal sperm cells NOAEL 320 ppm (69 mg/kg bw/day)	Agarwal et al. (1986a,b)
rat, F344 48 males/group	*gavage*, 13 days 0, 330, 1,000 or 3,000 mg/kg bw/day and a diet containing 2, 20 or 20 ppm zinc	Testis: dose-dependent tubular degeneration and atrophy from 1,000 mg/kg bw DEHP combined with low-zinc diet (2 ppm) NOAEL 330 mg/kg bw/day	Agarwal et al. (1986a)
rats, Sprague-Dawley 7-10 males/group	gavage, corn oil 5 days 0, 10, 100, 1,000 or 2,000 mg/kg bw/day at 1, 2, 3, 6 and 12 weeks of age neonatal exposure on days 6-10 0, 100, 200, 500 or 1,000 mg/kg bw/day	↓ absolute and relative testis weights at 1,000 mg/kg bw/day in 1, 2, 3,and 6-week old rats; ↓ Sertoli cell nuclei in 1-week-old rats and loss of spermatocytes in 2- and 3-week old rats; ↓ testis weight also in 6- and 12-week old rats at 2,000 mg/kg bw/day; fatalities in suckling rats at 2,000 mg/kg; NOAEL 100 mg/kg bw/day testis: ↓ number of Sertoli cells in adult rats at 500 and 1,000 mg/kg bw, no effect on fertility after mating to untreated females	Dostal et al. (1988)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
rat, F344 10 rats/sex/group	13 weeks, diet 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm (0, 80, 160, 320, 630, or 1,250 mg/kg/day)	↓bwg at 25,000 ppm testis atrophy from 12,500 ppm NOAEL 6,300 ppm (320 mg/kg/day)	NTP (1982)
rat, F344 50 rats/sex/group	103 weeks, diet 0, 6,000, or 12,000 ppm (0, 322, or 674 mg/kg/day [males])	↓bw at 12,000 ppm anterior pituitary: hypertrophy at 12,000 ppm (22/49 males, 45%) testis: seminiferous tubular degeneration at 6,000 ppm (2/44, 5%) and 12,000 ppm (43/48 males, 90%), histologically devoid of germinal epithelium and spermatocytes	NTP (1982)
rat, Wistar 6 males (25-day-old) per dose group	0, 50, 100, 250, or 500 mg/kg bw for 30 days	dose-dependent and significant ↑ LDH and GGT and ↓ SDH from 50 mg/kg bw; ↑ β-glucuronidase and ↓ acid phosphatase testis: marked destructive changes in the advanced germ cell layers and vacuolar degeneration at 250 and 500 mg/kg	Parmar et al. (1995)
rat, F344 70-85/sex/group recovery group: 55/sex	104 weeks, *diet* 0, 100, 500, 2500, or 12500 ppm (0, 5.8, 28.9, 146.6, or 789.0 mg/kg bw/day [males]; 0, 7.3, 36.1, 181.7, or 938.5 mg/kg bw/day [females] or 12500 ppm for 78 weeks, followed by a recovery period of 26 weeks	pituitary: ↑ castration cells (30/60 males) at 12500 ppm; testis: ↓ weight, ↑ incidence and severity of bilateral hypospermia at 12500 ppm; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 12500 ppm; changes in the testis and pituitary were not reversible upon cessation of exposure NOAEL for testicular effects 500 ppm (28.9 mg/kg bw/day)	Moore (1996)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
rat, Sprague-Dawley 10 rats/sex/group	13 weeks, *diet* 0, 5, 50, 500, or 5,000 ppm (0, 0.4, 3.7, 37.6, or 375.2 mg/kg bw/day [males])	testis: mild Sertoli cell vacuo-lation at 500 ppm (7/10); decreased absolute and relative testicular weight, mild to moderate Sertoli cell vacuolation, testicular atrophy and complete loss of spermatogenesis at 5,000 ppm (9/10), in-creased liver and kidney weights (all rats of both sexes), and mild histological changes of the thyroid at 5,000 ppm NOAEL 50 ppm (3.7 mg/kg bw/day)	Poon et al. (1997)
mouse, B6C3F1 70-85/sex/group; recovery group: 55/sex	104 weeks, *diet* 0, 100, 500, 1,500 or 6,000 ppm (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg bw/day [males] or 6,000 ppm followed by a recovery period of 26 weeks	testis: from 1,500 ppm ↓ weight, ↑ incidence and severity of bilateral hypospermia; epididymis: from 1,500 ppm ↑ immature or abnormal sperm forms and hypospermia; changes in testes partially reversible; NOAEL 500 ppm (98.5 mg/kg bw/day)	Moore (1997)
Developmental toxicity Studies			
Rat, Sprague-Dawley 17/males/group	3 generations via diet; 1.5, 100, 300, 1,000, 7,500 and 10,000 ppm (0.1, 0.5, 1.4, 4.8, 14, 46, 359, and 775 mg/kg/day	dose-dependent effets on numerous testis-related parameters. NOAEL for test.tox and dev. tox. 4.8 mg/kg/day, and 46 mg/kg/day for fertility	Wolfe et al, 2003
rat, Wistar 25 females/ group	*inhalation*, head-nose, gestation day 6-15 0, 0.01, 0.05, or 0.3 mg/litre (0, 10, 50, or 300 mg/m^3)	↓ number of live foetuses/dam and ↑ percentage of resorptions/dam at 50 mg/m^3; the effects showed, however, no dose-response relationship NOAEL for maternal and developmental toxicity 300 mg/m^3	Merkle et al. (1988)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
rat, F344/CrlBr 34-25 females/group	*Diet* 0, 0.5, 1.0, 1.5, or 2% gestation days 0-20	↓ maternal food intake and mean foetal bw from 0.5%; ↓ maternal bw gain, ↑ absolute and relative liver weights, ↓ foetal bw/litter from 1.0% ↑ number and percentage of resorptions, nonlive and affected implants/litter at 2%; NOAEL for maternal and developmental toxicity 0.5% (~357 mg/kg bw/day)	NTIS, 1984; Tyl et al. (1988)
rat, Wistar 9-10 females/group	*gavage*, oil 0, 40, 200 or 1,000 mg/kg bw/day on gestation days 6-15	↓ maternal bw and ↑ maternal relative kidney and liver weights at 1,000 mg/kg bw ↓ number of live foetuses/dam ↓ foetal body weights, ↑ number of malformed foetuses/dam (tail, brain, urinary tract, gonads, vertebral column, and sternum) at 1,000 mg/kg bw; NOAEL for maternal and developmental toxicity 200 mg/kg/day	BASF (1995); Hellwig et al. (1997)
mouse, 1-CR 30-31 females/group	*diet*; 0, 0.025, 0.05, 0.10 or 0.15% (0, 44, 91, 190.6 or 292.5 mg/kg bw/day); gestation days 0-17	↓ maternal body weight gain from 0.10% (mainly due to ↓ uterine weight, ↓ foetal body weight and number of live foetuses per litter); ↑ number and percent of resorptions, late foetal deaths, dead and malformed foetuses, and percent malformed foetuses/litter from 0.05% (open eyes, exophtalmia, exencephaly, short, constricted or no tail); visceral malformations and skeletal defects (fused and branched ribs, mis-alignement, and fused thoracic vertebral centra); NOAEL for maternal toxicity 0.05% (91 mg/kg bw/day) and for develop-mental toxicity 0.025% (44 mg/kg bw/day)	NTIS, 1984; Tyl et al. (1988)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
mouse, CD-1 15 females/dose group30 controls	*oral*, gavage 0, 40, 200 or 1,000 mg/kg bw/day gestation days 6-15	foetotoxic effects at 200 mg/kg bw/day ↓ number of viable foetuses ↑ number of resorptions and post-implantation losses at 1,000 mg/kg bw/day and also cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney abnormalities NOAEL 200 mg/kg bw for maternal toxicity and NOAEL 40 mg/kg bw/day for developmental toxicity	Huntingdon (1997)
Continuous breeding studies			
mouse, ICR 20 animals/sex/dose group, 40 control animals of each sex	*diet*, 98 days 0, 0.01, 0.1, or 0.3% (0, 20, 200 or 600 mg/kg bw/day)	dose-dependent ↓ in the number of litters and proportion of pups born alive from 0.1% (0.1%: 14/19 fertile, 0.3%: 0/18); ↑ absolute and relative liver weight (both sexes) and ↓ reproductive organ weights and atrophy of seminiferous tubules at 0.3%; no effect on bw NOAEL for maternal and developmental toxicity 20 and 600 mg/kg bw/day, respectively crossover mating trial: treated males and control females: 4/20 fertile; control males and treated females: 0/16 fertile	Lamb et al. (1987)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
Two-generation studies			
Rat, Sprague-Dawley 17/males/group	3 generations via diet; 1.5, 100, 300, 1,000, 7,500 and 10,000 ppm (0.1, 0.5, 1.4, 4.8, 14, 46, 359, and 775 mg/kg/day	dose-dependent effets on numerous testis-related parameters. NOAEL for test.tox and dev. tox. 4.8 mg/kg/day, and 46 mg/kg/day for fertility	Wolfe et al, 2003
Rat, Wistar, 25 animals/group	0, 1,000, 3,000 or 9,000 ppm DEHP via the diet (corresponding to approximately 0, 113, 340 or 1,088 mg/kg/day)	3,000 ppm; a reduced testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring. Minimal focal tubular atrophy also occurred at 1,000 ppm (113 mg/kg and day), which thus constitutes a conservatively chosen LOAEL	Schilling et al., 2001
Rat, Wistar 10 rats/sex/group	*diet, (range finding study)* 0, 1,000, 3,000 or 9,000 ppm (0, 110, 339 or 1,060 mg/kg bw/day)	↑ relative liver weight in F0 females from 1,000 ppm and in F0 males from 3,000 ppm (negative histopathology); ↓ food consumption, body weight, and body weight gain and ↑ post-implantation loss in females at 9,000 ppm; F1 pups :↓ number of delivered and live born pups and ↓ viability index neonatally at 9,000 ppm; loss of spermatocytes at 3,000 ppm (2/10) and 9,000 ppm (7/9); ↑ presence of areolas/nipple anlagen; retarded preputial separation and vaginal opening at 9,000 ppm; F1 parental animals : ↓ food consumption, body weight, and mortality in both sexes initially at 9,000 ppm and ↓ body weight gain in females; ↓ fertility, ↓ testicular and epididymal weight and size, atrophy of the testes, Leydig cell hyperplasia, interstitial oedema, and altered spermatogenesis and aspermia at 9,000 ppm; dose-related decrease of prostate weight from 1,000 ppm; F2 pups: ↑ number of still born pups from 3,000 ppm, ↓ number of delivered pups and mean number of pups/dam at 9,000 ppm;	Schilling et al. (1999)

Table 4.58 continued overleaf

Table 4.58 continued Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Results	References
mouse, CD-1 (number not specified)	*diet*, 0.01, 0.025, or 0.05% (0, 19, 48 or 95 mg/kg bw/day)	↑ prenatal mortality for F1-litters at 0.05% ↓ number of viable pups neonatally at 0.05% NOAEL for parental toxicity and F2-offspring: 0.05% (95 mg/kg bw/day) NOAEL for F1-offspring: 0.025% (48 mg/kg bw/d)	NTIS (1988)
Post-natal studies			
rat, Sprague-Dawley 10 males/group	*Gavage*, corn oil 5 days from the age of 1 week, 2 weeks, 3 weeks, 6 weeks, or 12 weeks 0, 10, 100, 1,000 or 2,000 mg/kg bw/day	two doses of 2,000 mg/kg bw were fatal for most pups in the three youngest age groups, ↓ bw for 6- and 12-week-old rats but no mortalities; 5 doses of 1,000 mg/kg bw: ↓ bw gain in 1-, 2-, and 3-week-old rats; ↑ absolute and relative liver weights at 100 mg/kg bw/day in all age groups (exept for 1-week-old rats) and in all age groups at higher dose levels; ↓ plasma cholesterol levels in weanling and adult rats from 1,000 mg/kg/day	Dostal et al. (1987b)

4.1.3 Risk characterisation for human health[7]

4.1.3.1 General considerations

4.1.3.1.1 Physical/chemical

DEHP is a liquid with a boiling point of 230°C with a relatively low volatility even at production and processing temperatures. DEHP has a low water solubility and a very high lipophility (log K_{OW}). DEHP is not readily flammable or explosive and is not expected to have oxidising properties.

4.1.3.1.2 Life-cycle stages

DEHP is widely used in many products in the society. The major use of DEHP is as a plasticiser in polymer products mainly in flexible PVC. DEHP occurs in many different life-cycle stages indicating both point and multiple sources of exposure. The main stages identified include:

- Production of DEHP
- Formulation of polymers
- Processing of polymers
- Formulation of non-polymers
- Processing of non-polymers
- Industrial end-use of products (articles) containing DEHP
- Private end-use of products (polymer *plus* non-polymer)
- Incineration of DEHP containing products
- Disposal on dump sites of DEHP containing products

Information is generally available for life cycle stages for production of DEHP and processing of flexible PVC but little information is available for formulation and processing of non-PVC and non-polymer uses. It was not possible to obtain information on the use of the total volume of DEHP produced or imported into the European Community, therefore it is recognised that not all life-cycle stages may have been covered for DEHP by this risk assessment.

4.1.3.1.3 Exposure

Exposure of different populations, occupational and non-occupational, to DEHP are listed in **Table 4.59**. The non-occupational population can be exposed from different sources: consumer products including medical equipment and indirectly via the environment. The non-occupational populations are also divided into sub-populations (e.g. adults, young/children) as the extent of

[7] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

exposure is expected to be different for sub-populations than for the population as a whole (see **Table 4.59**). Examination of the children separately from adults is considered necessary because several consumer products are age group specific (e.g. toys), and anatomical and physiological differences will affect the bio-availability of DEHP for these two age groups. In some scenarios, the sub-populations new-born (0-3 months of age) and infants (3-12 months of age) also occur.

Table 4.59 Populations and sub-populations exposed to DEHP

Population	Sub-population	Exposure route
Occupational	Adults	Inhalation, dermal
Consumer	Adults, children, infants and neonates.	oral, inhalation., dermal, i.v.
Man exposed indirectly via the environment	Adults, children, infants, new-born	oral, inhalation.

The different populations/sub-populations exposed by different routes of exposure are shown in **Table 4.60**. At a given time, exposure may occur either by a single or multiple pathways. Multiple pathways of exposure of a population/sub-population is considered to occur from different sources by different exposure routes (see **Table 4.60**). Exposure by multiple pathways is considered to be relevant when determining the body burden for each population/sub-population. The extent of exposure by multiple pathways is calculated as the sum of the highest exposure from each route from all sources during a day. Multiple pathways DEHP-intake is also calculated from recently measured data on urinary excretion of DEHP-metabolites in different populations.

The data presented in **Table 4.60** detail both the external and internal exposure. These data are either measured or modelled.

There are uncertainties about the contribution of exposure to DEHP adsorbed on particles and/or as an aerosol both in the work and residential environments. This may represent a significant exposure scenario, however, at present there does not exist sufficient data to make any firm conclusion.

Concerning the effects of protective measures on exposure of workers, few information are available to judge the relevance of such measures. Hence, it is not possible to consider the effect of protective measures on worker exposure in this RAR. For non-occupational populations/sub-populations, protective measures are not considered, because in cases where they may be recommended compliance cannot be assumed.

Since DEHP is diffusely spread in the environment and is present in a large number of products overall human exposure for a life time may be more relevant than just considering certain populations/sub-populations to specific sources. This may be achieved by estimating the total body burden by integrating exposure of all populations/sub-populations from all relevant sources and by all exposure routes. This can be estimated by considering multiple pathways of exposure and combined exposure (see **Table 4.61**).

It is recognised that not all exposed groups may have been identified since it was not possible to obtain extensive information on the exposure situations for DEHP in the European Community. Hence, not all exposure scenarios may have been covered for DEHP in this RAR. However this possible shortcoming has been overcome in adults by basing measurements of exposure on urinary excretion of DEHP metabolites.

Table 4.60 The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m^3)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Occupational								
Production of DEHP	5	530	650	460				990
Industrial use of DEHP	10	1060	420	300				1,360
Industrial end- use of products containing DEHP	10	1060	1300	928				1,988
Consumer								
- Adult								
"Indoor air" (building materials)	0.021[4]	4.4						4.4
Gloves			11.1	6.7				6.7
Car interior	0.021[4]	0.9						0.9
Multiple pathways of exposure		5.3		6.7				12
- Children								
"Indoor air" (building materials)	0.021[4]	22.4						22.4
Toys and child-care articles		-	2	9	1.6	200		209
Car interior	0.021[4]	2						2
Multiple pathways of exposure		24.4		9		200		231.4
Medical								
Haemodialysis (adults; long-term)							3,100	3,100
Blood transfusion (adults) (short-term)							8,500μg/kg bw/unit	

Table 4.60 continued overleaf

Table 4.60 continued The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m³)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Blood transfusion (new-borns) (short-term)							22,600μg/kg bw/unit	
Extracorporal oxogenation in infants (short-term)							140,000μg/kg bw/unit	
Peritoneal dialysis (long-term)							2	2
Clotting factors in haemophiliacs (adult; long-term)							30	30
Neonatal transfusion							1,700	1,700
Platelet and whole blood transfusion in infants (long-term)							6-75	6-75
Lifetime exposure (LADD) [1)]								
Infusion of platelets							1	1
Haemodialysis							99	99
Autopheresis							0.58	0.58
Man exposed indirectly via the environment[2]								
Adult. Total – regional (based on measured urinary excretion) [3]								17
Adult. Total –food, water and air, local (e.g. exposure via STP)								Table 4.13
Adult. Total -food, water and air, regional								1.7
Child. Total -food, water and air, local (e.g. exposure via STP)								Table 4.13

Table 4.60 continued overleaf

Table 4.60 continued The external and internal exposure of DEHP for different populations and examples of multiple pathways exposure to DEHP for three populations

Population	Inhalation	Inhalation	Dermal		Oral		Intravenous	Total multiple routes exposure
	External (mg/m^3)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	External (mg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)	Internal (μg/kg/day)
Child. Total -food, water and air, regional								18
Infants exposed via infant formulae and breast milk								
Infant formulae (0-3 months)						13		13
Infant formulae (3-12 months)						8		8
Breast milk (0-3 months)						21		21
Breast milk (3-12 months)						8		8

[1]LADD=lifetime average daily dose. Transfusion 2 years/lifetime, haemodialysis 15 years/lifetime

[2]The exposure indirectly via the environment goes via the oral route and inhalation (air). The oral exposure is the main exposure route (>99% of the total exposure via the environment).

[3] Daily regional exposure to DEHP has been estimated based on measured urinary excretion of DEHP-metabolites in a German population (Koch et al, 2003). The report calculates a 95th percentile intake value of 52.1 μg/kg/day based on excretion conversion factors of 5.5 and 7.4% for the two secondary metabolites of MEHP. When recalculated using the 'conversion factor' obtained from Koch et al (2003d), the 95th percentile intake value is 17 μg/kg/day.

[4] It should be noted that this figure is a truly worst case estimate.

Table 4.61 Combined exposure

Population	Source	i.	ii	iii.	iv.
Occupational		X			
Consumer	Toys and children's articles			X	X
	"Indoor air" (wall and floor covering)	X	X	X	X
	Gloves	X	X		
	Medical	(X)	(X)	(X)	(X)
Via the environment	Food, water and air	X	X	X	X
	Infant formulae				X
	Breast milk				X

Occupational: i. Adult.
Non-Occupational: ii. Adult; iii. Child; iv. Babies/infants;
"X" indicates exposure "(X)" indicates possible additional exposure

4.1.3.1.4 Toxicokinetics

There are a limited number of studies on the toxicokinetic behaviour of DEHP in man. These reports concern exposure by the oral, intravenous and inhalatory routes, however, they contain limited information. A single study performed by the dermal route is not considered reliable. Toxicokinetic studies in animals have been performed by the oral, inhalatory, dermal and parenteral routes. The majority of these studies have, however, been performed by the oral route and in three different strains of rat. Several studies with non-human primates, including old world- and new world apes, and mice have been conducted. In addition, there are several studies including other species. By the parenteral routes has been most studied in non-human primates, but there are few studies by the dermal and inhalation routes. In addition, *in vitro* studies have also contributed to the understanding of the toxicokinetics.

The majority of the toxicokinetic studies as a whole are mainly research orientated and published in the open litterature. Dose levels greatly varied between studies. The quality and relevance of these studies is variable and it was necessary to examine the database as a whole to derive conclusions.

Generally, DEHP (probably in the form of MEHP), is rapidly absorbed from the gastrointestinal tract following oral administration. The extent of absorption in rats, non-human primates and humans is around 50% for doses up to about 200 mg/kg bw. At higher doses, it appears that absorption in non-human primates is dose-limited in contrast to rodents.

For humans, information is not, however, available concerning the dependency of oral uptake on dose. Also, the extent of oral absorption at doses which humans are expected to be exposed is not known, although there are now some human toxicokinetic data. Absorption may be 100% at daily exposure levels. In addition, the oral absorption characteristics of human sub-populations e.g. age- and health dependent factors, are not known. Hence, for children it is considered appropriate to assume a 100% value for oral absorption. Because absorption via inhalation will comprise of respiratory and oral absorption, 100% bioavailability is also considered appropriate for children. Concerning dermal absorption, different bioavailability values were not selected for adults and children. The selected bioavailability values are summarised:

Summary of exposure route dependent systemic bioavailability	
Human exposure route	**Human systemic bioavailability (%)**
Oral	
Adults	50
Infants/children	100
Inhalation	
Adults	75
Infants/children	100
Dermal (free DEHP and in products)	
Adults	5
Infants/children	5
Parential routes	
All sub-populations	100

For rats a threshold was reached (450 mg/kg bw) above which there was a steady increase in the amount of unhydrolysed DEHP reaching the liver. In contrast, an absorption threshold could not be determined in $C3B6F_1$ mice for doses up to 1,000 mg/kg bw. There is no data available concerning possible absorption threshold in humans. Since, human exposure to DEHP is via inhalation, dermal, parential routes, one would expect that intact DEHP could reach the liver.

Limited data on toxicokinetics, following inhalation or dermal exposure, indicate that DEHP can be absorbed through the lungs whereas absorption through the skin appears to be limited. Following intra peritoneal injection most of the administered dose remains in the peritoneal cavity.

Distribution studies have mostly monitored total radioactivity rather than particular substances, so little is known about the tissue distribution of DEHP and its metabolites. The distribution studies indicate that the radioactivity from (^{14}C) DEHP is widely distributed in the body without evidence of accumulation in the tissues of rat. In a limited number of pigs and broiler hens, a lower degree of clearance of unlabelled DEHP is indicated. A comparative study of rats and marmosets showed similar distribution patterns in the two species (oral administration) whereas rats had higher tissue levels than marmosets. Thus, the difference in distribution between species is quantitative rather than qualitative.

The metabolism of DEHP involves several pathways and yields a variety of metabolites. The first step in the metabolism of DEHP is hydrolysis by lipases to MEHP and 2-EH. The lipases are found in all tissues surveyed but especially in the pancreas, indicating that most of DEHP hydrolysis occurs in the lumen of the small intestine, and that hydrolysis of absorbed intact DEHP can occur in the liver and blood. The absorption of DEHP in the intestine is increased following hydrolysis to MEHP.

MEHP is a relatively major component in urine of monkeys, guinea pigs and mice but was mostly not detected in rat urine. However, MEHP is present in plasma in all species tested.

The oxidative metabolism of MEHP in the liver begins with hydroxylation of the ethylhexyl side chain resulting in the formation of primary (ω-oxidation), and secondary ((ω-1)- and (ω-2)-oxidation) alcohols. These alcohols are then oxidised to diacids (ω-oxidation) or diketoacids ((ω-1)-oxidation), respectively. The diacids are apparently subject to α- or

β-oxidation at the ethyl- or hexylchain, respectively, in mitochondria and peroxisomes to yield shorter diacids. This process does not extend beyond the branch point in the ethylhexyl-chain. Generally, excluding the first hydrolysis step to MEHP, the metabolism of phthalate esters is qualitatively unaffected by the route of administration.

The first hydrolysis step, the hydrolysis of DEHP to MEHP, is common to all investigated species. One species difference related to the metabolism of DEHP seems to be that oxidative metabolism of MEHP plays a dominant role in rats but not in non-human primates. Following oral exposure approximately 75% of urinary metabolites consisting of dicarboxylic acids (mainly metabolites V and I) derived by ω-oxidation of the ethylhexyl chain. However, after *i.v.* administration there seems to be a more equal distribution between ω- and (ω-1)-oxidation. The predominant metabolites in non-human primates are MEHP and metabolite IX (a secondary alcohol) derived by (ω-1)-oxidation. Metabolites V and I are only minor metabolites in non-human primates. Regarding DEHP-metabolism, mice exhibit metabolic profiles in common with both rats and primates. For instance, metabolite I derived from ß-oxidative metabolism of DEHP is a major metabolite (pathway) in rodents, and occurs at relatively high levels in mice, but not in non-human primates. Also high levels of MEHP and metabolites from (ω-1)-oxidation are the major metabolites in the urine from non-human primates and mice, but not from rats. The limited human data indicate that human DEHP metabolism resembles that of other primates and of mice, with the exception of metabolite I in the latter case. However, regarding phase I metabolism of DEHP the species differences are quantitative rather than qualitative.

Glucuronidation is the major conjugation pathway identified in most species. In addition, ß-glucose conjugate was shown to be an alternative conjugation pathway in mice, but not in guinea pigs. Species differences have also been observed for glucuronidation since the fraction conjugated to glucuronic acid is absent in rats, low in hamsters, moderate in mice and guinea pigs. In primates, including humans, about 80% of the metabolites were glucuronides after intravenous administration, while for orally exposed humans about 65 and 99% of the metabolites, respectively, were glucuronides in two studies. The limited human data indicate that there are substantial inter individual differences e.g. polymorphism in the glucuronidation of some metabolites of DEHP, and of MEHP in particular. Mice that differ from rats in their ability to glucuronidate MEHP respond with the same types of toxic injury as do rats, indicating a possible partial independence of toxicity from the metabolic pathways in different species.

The elimination of DEHP largely depends on its metabolism and it might take 5-7 days to eliminate 80% of the radioactivity given as radiolabelled DEHP, either orally or intravenously. The half-life for DEHP and its metabolites was 3-5 days in the adipose tissue and 1-2 days in the liver. The elimination is most rapid in rats.

Many of the studies do not have complete recoveries, indicating that either biliary excretion and/or retention occur. Since many studies reveal that no significant retention was found in organs and tissues of laboratory test animals, biliary excretion appears to be an important excretion route. About 5-10% of the dose in rats was recovered from the bile in 24 hours after oral administration of DEHP, whereas about 24%, of the dose was recovered from the bile after intravenous administration of DEHP. In mice, biliary excretion seems to be one of the major route of exposure. In addition, metabolites are found in faeces from mice, rats and monkeys indicating that DEHP is absorbed, metabolised and excreted via the bile into the intestine. The extent of biliary excretion in humans is unknown. There are indications that resorption of radioactivity take place in the intestine of rats. Unfortunately, there is no data available on enterohepatic circulation of DEHP from other species than the rat, and the data on rats is very limited.

The radioactivity from (^{14}C) DEHP can cross the placenta barrier and distribute into foetal tissues. In addition, DEHP can be transferred through the milk from lactating rats to their pups. One study suggests that there are age-related differences since the extent of absorption and, hence, total systemic exposure to MEHP and its metabolites is higher in young rats than in old when DEHP is administered by gavage (Sjöberg et al., 1985c). Clearly higher blood levels were found in new-borns after blood transfusions, haemodialysis or treatment with platelet concentrates, compared to similarly exposed adults. Since the immature liver may have a lower metabolising capacity than that of older children and adults, infants and foetuses might be especially vulnerable to exposure of DEHP and MEHP.

In conclusion

The relative extent to which different metabolites are produced and excreted is very complex and may depend upon the specie, the age of the animal, sex, inter-individual differences, state of health, nutrition state, prior exposure to DEHP, the amount of DEHP administered, the administration route etc. With the exception of non-existing glucuronidation in rats there is no reason to suspect that functionally equivalent pathways for the metabolism of DEHP differ significantly in higher species.

The available data on the toxicokinetics of DEHP cannot explain the species differences in the DEHP-induced toxic effects, and are consistently not adequate to support any conclusion on the relevance or irrelevance for humans of the DEHP-induced toxic effects in experimental animals.

4.1.3.1.5 Effects data

The assessment of the hazardous properties of DEHP is based on animal data as no significant human data are available. Numerous studies on the toxicity of DEHP have been conducted both in experimental animals and *in vitro.* A number of the available studies have been omitted from the risk assessment report because of limited quality of these studies or relevance to the risk assessment. Repeated dose kidney and testis toxicity, and effects on development and fertility are considered to be critical endpoints in the risk assessment of DEHP (see **Table 4.62** and **Table 4.63**).

Acute toxicity studies of good quality indicate low acute toxicity of DEHP. Oral LD_{50} > 20,000 mg/kg bw in rats and > 10,000 mg/kg bw in mice and an inhalation LC_{50} of about 10,600 mg/m^3 for 4 hours in rats have been reported. Although there are no adequate acute dermal toxicity data, low dermal absorption is suggestive of a low acute dermal toxicity. Following intravenous administration of DEHP in rats effects on the cardiovascular system and lungs were observed. The studies are considered inadequate for risk characterisation, however, if these effects are substantiated they are considered of concern for vulnerable populations such as patients and children.

Animal studies performed according to current standards have shown a slight skin and eye irritation after administration of DEHP and limited information suggests a potential for DEHP to induce lung lesions following acute inhalation. DEHP is not corrosive to the skin or eyes.

DEHP has not been found to induce skin sensitisation in animals. Based on the available data there are no clear evidence that DEHP causes respiratory sensitisation. However, there are some indications that bronchial obstruction and asthma may increase in the presence of DEHP and other plasticiser in PVC products found in the indoor environment (Jaakkola et al 1999; Øie et al., 1997).

Concerning repeated dose toxicity limited human data are available. There is, however, information that toxic damage of the lungs in preterm infants artificially ventilated with PVC respiratory tubes may be causally related to inhalation of DEHP. The estimated inhalative exposure ranged between 1µg/h – 4,200µg/h DEHP. Other studies, one morbidity and one mortality study, and three epidemiological studies conducted on workers exposed to DEHP and other phthalate esters are considered inadequate with respect to the risk assessment.

In experimental animals a few inhalation studies are available. However, due to concerns about the inadequacy of the reported data e.g. insufficient dosing these studies are considered inadequate for risk assessment. Also the only study available following dermal exposure to DEHP is inadequate for risk assessment.

Numerous studies have investigated the toxicity of DEHP following repeated oral administration to experimental animals, preferably rats. Many of these studies are comparable to guideline studies and conducted in conformity with GLP. Critical organs for DEHP induced toxicity in laboratory animals are the testis, kidney, and liver.

Testicular effects are discussed in more detail in Section 4.1.2.10. In repeated dose studies, a NOAEL of 4.8 mg/kg/day (100 ppm) is obtained for testicular effects identified in a guideline three-generation reproductive toxicity study (Wolfe et al., 2003). A 90-day oral study in rats gave a NOAEL of 3.7 mg/kg/day (50 ppm) (Poon et al., 1997). However, as there remains some doubts as to the toxicological significance of the sertoli cell vacuolisation observed in the Poon study, a NOAEL of 4.8 mg/kg/day (100 ppm) is chosen from the Wolfe study (2003) for the risk characterisation, based on occurrence of small male reproductive organs (testis/epididymes/seminal vesicles) and minimal testis athrophy (exceeding those of the current controls as well as historical control groups) at 300 ppm and above. This may be considered a conservative choice of NOAEL.

The effects on the kidneys include: increased absolute and relative kidney weights, increased incidence and severity of mineralisation of the renal papilla, increased incidence and/or severity of tubule cell pigment, and increased incidence and/or severity of chronic progressive nephropathy. The majority of these changes were observed in both sexes, in different species following different exposure time. In long-term studies in rats and mice, there was no indication that DEHP-related changes in the kidney were reversible upon cessation of DEHP-exposure. The lowest NOAEL for kidney toxicity is 500 ppm DEHP in the diet (corresponding to 28.9 mg/kg/day in the males and 36.1 mg/kg/day in the females) derived from a well-performed 104-week-study in rats (Moore 1996) and based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL = 146.6 mg/kg bw/day). More severe kidney lesions were observed at the highest dose level.

In the liver, the most striking effects observed are hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation, and hepatocellular tumours.

Marked species differences are apparent in response to the hepatotoxic effects of DEHP and other peroxisome proliferators (PPs). Rats and mice are very sensitive whereas hamsters, guinea pigs, and monkeys appear to be relatively insensitive or non-responsive at dose levels that produce a marked response in rats. It has been suggested that there may be an association between peroxisome proliferation and the occurrence of liver tumours in rats and mice after long-term exposure. Recent investigations have demonstrated that activation of the peroxisome proliferator-activated receptors (PPAR-α) is required for induction of the different PPs-induced liver effects observed in experimental animals (Peters et al., 1997; Ward et al., 1998; Cattley et

al., 1998). The low sensitivity of human liver to the hepatotoxic effects of PPs could be explained by the low level of PPAR-α found in human liver and genetic variations that render the human PPAR-α less active as compared to PPAR-α in rodent liver (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt et al., 1999).

Most recently, a Working Group of the "International Agency for Research on Cancer" (IARC) have concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α) is not relevant to humans. Therefore, and based on the overall evaluation of the available data, the DEHP-induced hepatotoxic effects in rats and mice will not be considered in the present Risk Assessment Report on DEHP.

The data available on repeated dose toxicity (not including reproductive effects) do not suggest a classification of DEHP according to EU criteria.

Concerning genotoxicity of DEHP, several different short-term tests, comparable to guideline studies and performed according to GLP, were available. The results have been negative in the majority of the *in vitro* and *in vivo* studies performed with DEHP, and its metabolites for detection of gene mutation, DNA damage, and chromosomal effects. The more conclusive positive results were obtained in the test systems for detection of cell transformation, induction of aneuploidy, and cell proliferation which are also sensitive to several non-mutagenic substances such as tumour promoters and/or peroxisome proliferators. Taken together all the results, both negative and positive, DEHP and its major metabolites could be considered to be non-mutagenic substances. No adequate studies to assess the mutagenicity of DEHP to humans are available.

No relevant human data on carcinogenicity is available. In experimental animals, the only inhalation study available (Schmezer et al., 1988) is in hamster and is regarded as inadequate for risk assessment due to insufficient dosing (only one dose was used and MTD was not reached). Following oral exposure, four long-term carcinogenicity studies (Moore 1996, 1997; NTP studies, 1982) performed in rats and mice are of good quality and are considered adequate for evaluation of carcinogenicity of DEHP in experimental animals. DEHP shows clear evidence of hepatocarcinogenicity in both sexes of rats and mice in the four studies and an increase in the incidence of mononuclear cell leukaemia in male rats in one study (Moore, 1996). Additionally, an increase in the incidence of testicular interstitial cell tumours (LC tumours) was observed in Sprague-Dawley rats exposed for DEHP, 30, 95 and 300 mg/kg, in the diet, in a lifelong study published as an abstract (Berger 1995). However, these indications have not been confirmed in later studies (e.g. multigeneration studies).

The results of the animal studies clearly show that DEHP is carcinogenic in rats and mice. However, there is a plausible mechanism for the PPs-induced hepatocarcinogenicity in rodents (activation of PPARα) and there is evidence showing that humans are less sensitive to the hepatotoxic effects of PPs by the suggested mechanism. Therefore, the relevance for humans of the liver tumours in rodents induced by DEHP, a weak PPs, is regarded to be neglgible. Also the relevance of the DEHP-induced MCL in F344 rats is questionable. On the other hand, the induction of LC tumours in rats exposed for DEHP may be relevant to humans, however, an evaluation of the orignal data of Berger (1995), reported in an abstract, is necessary before concluding any possible carcinogenic risk of DEHP. No classification for carcinogenicity is proposed.

DEHP has been shown to influence both fertility and development. Testicular effects have been observed both in several repeated dose toxicity studies and in reproductive toxicity studies in

male rats. Both *in vivo* and *in vitro* experiments have demonstrated that the Sertoli cell is the main target of DEHP induced testicular toxicity producing subsequent germ cell depletion.

MEHP is believed to be the active metabolite of DEHP affecting testes and reproductive functions both *in vivo* and *in vitro*. The possible role of other metabolites is, however, not fully elucidated.

Testicular effects have been observed in several repeated dose toxicity studies in rats, mice, ferrets, **(**Gray et al., 1977; NTP, 1982; ICI, 1982b; CMA, 1984b,c; Ganning et al., 1990; Eastman Kodak, 1992a; Moore, 1996; Poon et al., 1997; Lamb et al., 1987; NTP, 1982; Moore, 1997; Lake et al., 1976; 1986, Gray et al., 1982, Schilling at al., 2001, Wolfe et al., 2003). In addition, minor effects were obsereved in hamster exposed to DEHP and more severe effects induced by MEHP (Gray et al., 1982). In the available studies marmosets were not sensitive to DEHP(Kurata et al., 1995; 1996; 1998). No studies on testicular effects in rabbits are available.

In the rat, the lowest NOAEL for testicular effects is 50 ppm (3.7 mg DEHP/kg bw/day, administered in the diet) identified in a 90 day guideline study and based on a high and dose-dependent incidence of Sertoli cell vacuolation at the next dose level (500 ppm equivalent to 37.6 mg/kg bw/day) (Poon et al., 1997). At the highest dose level (5,000 ppm equivalent to 375.2 mg/kg bw/day) also a high incidence of atrophy of the seminiferous tubules with complete loss of spermatogenesis was found in addition to the Sertoli cell vacuolation. As this study is of crucial importance, it has been peer reviewed and evaluated by a panel of experts in the area of reproductive toxicology. However, as there remains some doubts as to the toxicological significance of the sertoli cell vacuolisation observed in the Poon study, a NOAEL of 4.8 mg/kg/day (100 ppm) is chosen for the risk characterisation, based on occurrence of small male reproductive organs (testis/epididymes/seminal vesicles) and minimal testis athrophy (exceeding those of the current controls as well as historical control groups) at 300 ppm and above in a three-generation rat study (Wolfe et al., 2003).

Developing and prepubertal rats have been found to be much more sensitive to exposure to DEHP than adults (Gray and Butterworth, 1980; Sjöberg et al., 1985c; Arcadi et al., 1998; Wolfe et al., 2003). In the 90-day study conducted by Poon et al., (1997), rats were exposed both before and mainly after sexual maturation. However, the study did not discern an age-dependent effect, therefore, the results of this study are considered relevant for both young and adult males. Furthermore, humans are exposed to DEHP for their whole lifetime, i.e prenatally to death, via the environment, consumer products and medical devices. In addition, occupational exposure may occur. Since the study conducted by Wolfe et al (2003) was a three-generation study, involving exposure through all life stages, the Wolfe study covers all stages of human exposure.

Based on the available data, which varies in both the study designs and number of animals included, testicular effects have been demonstrated in both male rodents and non-rodents: rat (NOAEL = 3.7 and 4.8 mg/kg bw/day) mouse (NOAEL = 98.5 mg/kg bw/day), and the ferret (LOAEL = 1,200 mg/kg/day) (Wolfe et al., 2003; Poon et al., 1997; Moore, 1997; Lake et al., 1976). In addition, minor effects were observed in hamster exposed to DEHP and more severe effcets induced by MEHP (Gray et al., 1982). In the available studies with marmosets testicular toxicity has not been observed after treatment with DEHP (Kurata et al., 1995; 1996; 1998). The reasons for the differences in study results has been suggested to concern toxicokinetic considerations and altered zinc homeostasis. Moreover, other factors such as animal age, study design, animal model selection have to also be considered. For instance, marmosets which are new-world monkeys vary in their metabolic pathways and capacities and are not as closely related to humans as are cynomolgus and Rhesus monkeys (old-world monkeys). This is elaborated in the course of studies on 40 medical drugs (Caldwell 1977, 1979, 1985, 1979a,b;

Siddall, 1978). The mechanism(s) and/or factors that cause the observed differences in the DEHP-induced testicular toxicity have not been substantiated. Based on the available animal data it is not possible to definitely conclude the relevance of these differences in humans. However, in the limited toxicokinetic data in humans, MEHP, the testicular toxicant, is formed following exposure to DEHP. Therefore, DEHP-induced testicular effects observed in animal studies are considered relevant for humans, and the NOAEL of 4.8 mg/kg/day (Wolfe et al., 2003) is selected for the risk characterisation of humans.

Effects on male fertility have been observed in mice and rats. In mice, DEHP adversely affects the number of fertile matings. In a continuous breeding study an oral NOAEL of 0.01% in the diet (20 mg/kg bw/day) was identified for fertility (Lamb et al, 1987). In male rats, endocrine and gonadal effects were observed (Agarawal et al, 1986a,b).

Effects on developmental toxicity have been observed in several studies. The rat has been shown to be the most sensitive species to DEHP-induced malformations. Irreversible testicular damage in the absence of obvious effects on the dams was shown in male pups exposed *in utero* and during suckling at very low dose levels (LOAEL = 3.5 mg/kg bw/day) (Arcadi et al., 1998). However, there are some limitations in this study due to dosing uncertainties and therefore it is not used for risk characterisation. Effects on the male reproductive system, partly induced during the gestational period, were also observed in a three-generation study with a NOAEL of 4.8 mg/kg/day (Wolfe et al., 2003). Because of some uncertainties with regard to the dosing in the Arcadi study, the risk characterisation will be based on the Wolfe study. In mice, DEHP is embryotoxic and teratogenic (NOAEL = 20 mg/kg bw/day) at oral dose levels below those producing observable evidence of toxicity to the dams (600 mg/kg bw/day) (Lamb et al., 1987).

Both *in vivo* and *in vitro* study results indicate that DEHP can interfere with the endocrine function and also influence the sexual differentiation. Due to the effects on the Leydig cells as measured by a decreased testosterone output, it cannot be excluded that DEHP may exert an antiandrogen effect (Jones et al., 1993). Recently performed *in vivo* studies in rats support the hypothesis that exposure to phthalates may be provoked by an antiandrogen mechanism (Gray et al., 1999).

Developing and prepubertal rats have been found to be much more sensitive to exposure to DEHP than adults (Gray and Butterworth, 1980; Sjöberg et al., 1985c; Sjöberg et al., 1986a,b; Gray and Gangolli, 1986; Dostal et al., 1988; Wolfe et al., 2003). The younger animals respond to a much lower dose or produce a more serious lesion with a comparable dose on a mg/kg/day basis. In some instances, the onset for the production of the lesion is also more rapid.

To conclude the reproduction toxicity, the available data in experimental animals are of concern for humans. The effects on testis, fertility, and development, observed in different animal species and at relatively low dose levels, are considered to be relevant to humans. Developing and prepubertal rats have been found to be more sensitive to exposure to DEHP than adults. Also the results of different studies indicate that DEHP can interfere with the endocrine function and also influence the sexual differentiation. Additionally, animal data have shown that DEHP and its metabolites can be transferred to pups via mothers milk in concentrations sufficient to cause toxicity. DEHP has also been found in infant formulas and mothers milk. According to the EEC criteria for classification and labelling of dangerous substances DEHP is classified in Category 2 both as toxic for reproduction and for development (R60-61).

Table 4.62 Studies showing the critical endpoints

Species	Study Protocol; Quality	Effects observed at LOAEL	LOAEL	NOAEL	Ref.
Repeated-dose toxicity					
Rat, F-344, males and females	Diet, 2 years; GLP, comparable to guideline study	Both sexes:↑ absolute and relative kidney weight More severe kidney lesions were observed at the highest dose level	147 mg/kg bw/day	29 mg/kg bw/day	Moore (1996)
Reproductive toxicity					
Rat, Sprague-Dawley, males and females	Diet, 3-generation guideline study	Testicular toxicity as well as Developmental toxicity: increased incidences of small testes, epididymes, and seminal vesicles, as well as cases of minimal testes atrophy. The toxicity was aggravated by exppsure during the gestational/pup-period	14 mg/kg/day	4.8 mg/kg/day	Wolfe et al. (2003)
Mouse, CD-1, males and females	Diet, continous breeding study; GLP, comparable to guideline study	Fertility ↓ (dose-dependent ↓ in the number of litters) and ↓ proportion of live pups; crossover matings showed that both sexes were affected	200 mg/kg bw/day	20 mg/kg bw/day	Lamb et al. (1987)

4.1.3.1.6 Issues considered when calculating MOS and/or determining the conclusions

To conduct the risk characterisation for different human populations, it is necessary to compare human internal (systemic) exposure for different routes with the systemic (internal) NOAELs from toxicity studies in experimental animals. Thus, the exposure is expressed as the internal, systemic dose, after considering different absorption degrees via the different routes. In calculation of systemic NOAELs, it is necessary to compensate for age differences in oral uptake. In the cases where the NOAELs come from studies in adult animals (kidney toxicity and fertility), calculation of systemic NOEALs is based on only a 50% absorption in adult animals (giving systemic NOAELs that are half of the NOAELs given by the actual studies). However, for the NOAELs coming from the 3-generation study (testicular toxicity and developmental toxicity), no recalculation to a systemic NOAEL is needed as a major part of the exposure occurs during life stages with an assumed oral absorption of 100%. An assessment factor of 10 is used for potential species differences. Assessment factors of 3 and 10 are used for potential intraspecies differences for the worker and general population, respectively. Consideration is also given to the severity of the effect. There is no need to correct for duration as the NOAELs are based on chronic or multi-generation studies.

The nature of the DEHP-induced toxic effects is considered serious. Effects on the testis, fertility, development, and kidney (repeated dose toxicity) are considered to be the most critical effects. Severe and irreversible testicular injury was induced in rats exposed to low oral dose levels of DEHP in three different studies (Wolfe et al., 2003; Poon et al., 1977; Arcadi et al., 1999, 1998). Also severe developmental effects were observed in rats and mice in the absence of

maternal toxicity (Wolfe et al., 2003; Arcadi et al., 1998; Lamb et al., 1987). MOS is determined based on all critical effects for adults, but for children the developmental effect is not considered as it is not relevant. Thus, consideration is given to the fact that the endocrine effects (e.g. underlaying the testicular toxicity) are very serious effects, and that the sensitivity to this effect is highest during gestation and the first few months after birth when the most sensitive systems are still developing. This is to some extent also considered when it comes to developmental effects and adult worker exposure. Adults and older children are otherwise treated equally. However, based on the severity and the risk for combined exposure (see **Table 4.61**), the MOS cut-off values (minMOS) are increased in 3-12 months old infants (200), with a further increase in newborn (< 3 months of age) (250). However, all member states do not agree that newborns/infants need a higher margin of safety in this particular case. It is also considered that the choice of NOAEL in the Wolfe study may be considered a conservative choice.

It has to be acknowledged that there is also a potential for exposure of infants to other phthalates that are toxic to reproduction via similar mechanisms of action as DEHP. As an illustration of potential for exposure, indoor dust in the UK contains dibutylphthalate (DBP, median 53 µg/g dust) and butylbenzylphthalate (median 24 µg/g dust) (Santillo et al., 2003). Their monoester metabolites are also found in urine of American children (Brock et al., 2002). The presence of monobutylphthalate in breast milk has also been indicated. Similar and possibly additive effects of DEHP and DBP has been indicated in an abstract by Foster et al (2002). However, the possible impact of co-exposure to other phthalates has not been considered in this risk assessment of DEHP.

Because of the low degree of acute toxicity, lack of irritation and sensitising effects and lack of evidence for mutagenicity, these endpoints are not considered further in this assessment. Also the carcinogenicity will not be considered in the risk characterisation as the liver tumours observed in rats and mice were regarded as irrelevant for humans. Data on other tumour types is not adequate for risk characterisation.

Both human external and internal exposure values used for the determination are presented in **Table 4.60**. Values used in EUSES are presented in **Table 4.13**. MOSs are derived for each population/sub-population and exposure scenarios are considered to be worst-case. Values have been derived for exposure by single and multiple pathways. Exposure by multiple pathways is considered to reflect the highest exposure for a population/sub-population. No attempt has been made to quantitatively estimate MOS for combined exposure (see **Table 4.61**), but it is considered in setting the minMOS for babies (infants and newborns).

A few animal inhalation studies are available, however, due to concerns about the inadequacy of the reported data e.g. insufficient dosing, these studies are considered inadequate for risk assessment. Therefore, NOAELs derived from animal oral studies were used to calculate MOS as these were considered to be more reliable than the inhalation studies.

Additionally, the following factors were considered:

- DEHP is produced and used widely in the EU, however, measured exposure data are limited.
- Not all exposure scenarios in the EU may have been identified. This is of particular importance when summarying the exposure from different sources such as “Multiple pathways of exposure” and “Combined exposure”, since the total exposure may be underestimated. However, this possible shortcoming has been overcome in adults by basing measurements of exposure on urinary excretion of DEHP metabolites.

- Interactive effects due to exposure to a mixture of phthalates either simultaneously or sequentially cannot be ruled out.

Table 4.63 Summary of NOAELs used for the calculation of MOS (also see Table 4.62)

Effect	Route	LOAEL (mg/kg bw/d)	NOAEL (mg/kg bw/d)	$NOAEL_{systemic}$[1] (mg/kg bw/d)
RDT (effects on kidney)[2]	Oral	147	29	14.5
Testicular [3]	Oral	14	4.8	4.8
Fertility[4]	Oral	200	20	10
Developmental[3]	Oral	14	4.8	4.8

1) Correction of NOAEL for 50% oral absorption in adult rats
2) 2-year oral study in rats (Moore 1996)
3) Three-generation oral study in rats (Wolfe et al., 2003). No correction of the NOAEL is needed as the study is considered to directly give a systemic NOAEL (mainly exposure of young animals with 100% absorption)
4) Continuous breeding study in mice (Lamb *et al*.,1987)

4.1.3.2 Risk characterisation of workers

Based upon the evaluated information in Section 4.1.1.1 the type of work tasks that may lead to the highest exposures to DEHP are considered to be during the industrial use of DEHP. This exposure is expected to be higher than for the production of DEHP or industrial end-use of products containing DEHP. Little information is, however, available for the industrial use of DEHP for non-PVC polymer products, and Industrial end-use of semi- and end-products containing DEHP

The exposure scenarios considered for workers in this RAR concern exposure to DEHP from:

- production of DEHP
- industrial use of DEHP
- industrial end-use of products containing DEHP

Measured data of dermal exposure for all the occupational exposure scenarios is not available. Hence, for the scenarios of production of DEHP and industrial use of DEHP, worst-case exposure has been calculated using the EASE model for dermal exposure.

For the scenario of industrial end-use of products containing DEHP, no measured data or background information useful for calculation of the exposure is available. Hence, the following assumptions are made, supposing situations with relatively high temperature, aerosol generating processes and considerable skin contact.

During industrial use, workers are expected to be mainly exposed to DEHP via inhalation and dermal routes. Exposure is also considered to be mainly to DEHP aerosol but exposure to gaseous DEHP may also occur. Oral exposure to DEHP is considered to be potentially low and therefore is not considered.

MOSs presented here have been derived based on the following scenarios (see **Table 4.64**).

Table 4.64 Calculation of MOS for workers exposed to DEHP. Human internal exposure by inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
Production of DEHP					
Inhalation					
RDT	0.530	29	14.5	28	(iii)
Testicular	0.530	4.8	4.8	9	(iii)
Fertility	0.530	20	10	19	(iii)
Developmental	0.530	4.8	4.8	9	(iii)
Dermal					
RDT	0.460	29	14.5	31	(ii)
Testicular	0.460	4.8	4.8	10	(iii)
Fertility	0.460	20	10	22	(iii)
Developmental	0.460	4.8	4.8	10	(iii)
Multiple Routes of Exposure (inhalation + dermal)					
RDT	0.99	29	14.5	14	(iii)
Testicular	0.99	4.8	4.8	5	(iii)
Fertility	0.99	20	10	10	(iii)
Developmental	0.99	4.8	4.8	5	(iii)
Industrial use of DEHP					
Inhalation					
RDT	1.06	29	14.5	13	(iii)
Testicular	1.06	4.8	4.8	5	(iii)
Fertility	1.06	20	10	9	(iii)
Developmental	1.06	4.8	4.8	5	(iii)
Dermal					
RDT	0.3	29	14.5	48	(ii)
Testicular	0.3	4.8	4.8	16	(iii)
Fertility	0.3	20	10	33	(ii)
Developmental	0.3	4.8	4.8	16	(iii)
Multiple Routes of Exposure (inhalation + dermal)					
RDT	1.36	29	14.5	11	(iii)
Testicular	1.36	4.8	4.8	4	(iii)
Fertility	1.36	20	10	7	(iii)
Developmental	1.36	4.8	4.8	4	(iii)

Table 4.64 continued overleaf

Table 4.64 continued Calculation of MOS for workers exposed to DEHP. Human internal exposure by inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
Industrial end use of products containing DEHP					
Inhalation:					
RDT	1.06	29	14.5	14.5	(iii)
Testicular	1.06	4.8	4.8	5	(iii)
Fertility	1.06	20	10	10	(iii)
Developmental	1.06	4.8	4.8	5	(iii)
Dermal					
RDT	0.928	29	14.5	16	(iii)
Testicular	0.928	4.8	4.8	5	(iii)
Fertility	0.928	20	10	10	(iii)
Developmental	0.928	4.8	4.8	5	(iii)
Multiple Routes of Exposure (inhalation + dermal)					
RDT	2	29	14.5	7	(iii)
Testicular	2	4.8	4.8	2	(iii)
Fertility	2	20	10	5	(iii)
Developmental	2	4.8	4.8	2	(iii)

1) Internal exposure based on measured and modelled data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular and developmental toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

4.1.3.2.1 Factors considered when calculating MOS and or determining conclusion

Factors common for all exposure scenarios are presented under "Issues considered when calculating MOS and/or determining the conclusions"; Section 4.1.3.1. In addition, factors specific for occupational exposure have also to be considered:

- Measured exposure data are limited to certain industrial practices and not representative for all EU member states
- PPE is not considered as a consistently reliable risk reduction measure for use in the risk assessment of DEHP

The resulting minMOSs that the MOS-values will be compared with is approximately 30; slightly higher minMOS are used for developmental toxicity and slightly lower for the kidney toxicity, based on the severity of the effects.

Summary and conclusion

There is concern with regard to testicular effects, fertility, toxicity to kidneys, on repeated exposure and developmental toxicity for workers as a consequence of inhalation and dermal exposure. There is no concern for the acute toxicity, irritation and sensitising effects, carcinogenicity, and mutagenicity.

Conclusion (iii).

4.1.3.3 Risk characterisation of consumers

Adults and children are considered separately as two different sub-populations because several consumer products are intended for specific age groups (e.g. toys), and anatomical and physiological differences between adults and the children can significantly affect the bio-availability of DEHP in these two groups. In addition, medical devices are considered separately as these products are not readily available to the general public and the route of exposure is mainly intravenous.

4.1.3.3.1 Risk characterisation of adult consumers

Exposure scenarios considered important for the adult consumer concern exposure to DEHP from:

- In-door air (building materials)
- Gloves
- Car interiors
- Multiple Pathways of Exposure from the above

The information used is based on modelling of concentrations of DEHP in the indoor air and absorption from gloves. In the air DEHP may be present as a gas, an aerosol and/or bound to particles.

MOSs presented here have been derived based on the following scenarios (see **Table 4.65**).

Table 4.65 Calculation of MOS for adult consumers exposed to DEHP. Human internal exposure by inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
1. Indoor air (building materials)					
Inhalation					
RDT	0.0044	29	14.5	3,295	(ii)
Testicular	0.0044	4.8	4.8	1,091	(ii)
Fertility	0.0044	20	10	2,272	(ii)
Developmental	0.0044	4.8	4.8	1,091	(ii)

Table 4.65 continued overleaf

Table 4.65 continued Calculation of MOS for adult consumers exposed to DEHP. Human internal exposure by inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{(NOAEL, Systemic)}$)[4]	Conclusion
2. Gloves					
Dermal					
RDT	0.0067	29	14.5	2,164	(ii)
Testicular	0.0067	4.8	4.8	716	(ii)
Fertility	0.0067	20	10	1,492	(ii)
Developmental	0.0067	4.8	4.8	716	(ii)
3. Car interiors					
Inhalation					
RDT	0.0009	29	14.5	16,111	(ii)
Testicular	0.0009	4.8	4.8	5,333	(ii)
Fertility	0.0009	20	10	11,111	(ii)
Developmental	0.0009	4.8	4.8	5,333	(ii)
3. Car interiors					
Multiple Pathways of Exposure (inhalation + dermal, i.e. scenario 1 + 2 + 3)					
RDT	0.012	29	14.5	1,208	(ii)
Testicular	0.012	4.8	4.8	400	(ii)
Fertility	0.012	20	10	833	(ii)
Developmental	0.012	4.8	4.8	400	(ii)

1) Internal exposure based on measured data (see Table 4.60). It should be noted that the indoor air exposure estimate is a worst case estimate.
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular and developmental toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Factors considered when calculating MOS and or determining conclusion

See factors common for all exposure scenarios, under "Issues considered when calculating MOS and/or determining the conclusions" Section 4.1.3.1. A MOS > 100 is considered an acceptable cut-off for all endpoints because of the conservative nature of the exposure values.

Summary and conclusion

There is no concern for exposure from indoor air, gloves, car interiors and multiple pathways of exposure for all toxicological endpoints studied.
Conclusion (ii).

4.1.3.3.2 Risk characterisation of children consumers

Exposure scenarios considered important for children, concern exposure to DEHP from:

- Indoor air (building material)
- Toys and child-care articles
- Car interiors
- Multiple Pathways of Exposure

The information used is based on measurements of DEHP in artificial and human saliva for toys and baby equipment and an estimated value for indoor air. In the air DEHP may be present as a gas and bound to particles. MOSs presented here have been derived based on the following scenarios (see **Table 4.66**):

Table 4.66 Calculation of MOS for children consumers exposed to DEHP. Human internal exposure by oral, inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
1. Indoor air (building materials)					
Inhalation					
RDT	0.022	29	14.5	659	(ii)
Testicular	0.022	4.8	4.8	218	(ii)
Fertility	0.022	20	10	455	(ii)
2. Toys and child-care articles					
Oral					
RDT	0.2	29	14.5	72	(iii)
Testicular	0.2	4.8	4.8	24	(iii)
Fertility	0.2	20	10	50	(iii)
Dermal					
RDT	0.009	29	14.5	1611	(ii)
Testicular	0.009	4.8	4.8	533	(ii)
Fertility	0.009	20	10	1111	(ii)
3. Car interiors					
Inhalation					
RDT	0.002	29	14.5	7250	(ii)
Testicular	0.002	4.8	4.8	2,400	(ii)
Fertility	0.002	20	10	5000	(ii)

Table 4.66 continued overleaf

Table 4.66 continued Calculation of MOS for children consumers exposed to DEHP. Human internal exposure by oral, inhalation and dermal routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
3. Car interiors					
Multiple Pathways of Exposure (oral + inhalation + dermal, i.e. scenario 1 + 2 + 3)					
RDT	0.234	29	14.5	63	(iii)
Testicular	0.234	4.8	4.8	21	(iii)
Fertility	0.234	20	10	43	(iii)

1) Internal exposure based on measured data (see Table 4.60) . It should be noted that the indoor air exposure estimate is a worst case estimate.
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAEL for testicular toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Factors considered when calculating MOS and or determining conclusion

Factors common for all exposure scenarios are presented under "Issues considered when calculating MOS and/or determining the conclusions" (Section 4.1.3.1).

Concerning human exposure, differences in oral and inhalation uptake between children and adults has been accounted for as the internal exposures has been estimated based on different bioavailability factors for children in comparison with adults (GI-tract: 100% in comparison with 50%; and, inhalation: 100% in comparison with 75%). For dermal exposure, internal exposure of children has been estimated based on the same bioavailability factor (5%) as used for adults. We believe, however, that this value may underestimate dermal uptake in children compared with adults and, hence, warrants a higher cut-off for all toxicological endpoints.

Concerning different toxicological end-points, different cut-offs for children are selected when the derived MOS values do not clearly indicate a **conclusion (ii)** or **conclusion (iii)**, or clarification is required:

- A MOS > 100 is considered an acceptable cut-off for effects on kidney (RDT) because the study is long-term (two-years).
- A cut-off higher than 100-200 (depending on age; 200 for infants, 100 for children) is recommended for effects on the testes and fertility because:

  1) Testicular toxicity is a very serious end-point, especially in infant (sucking on toys and child-care articles), as this is a very sensitive life-stage. Rats have been shown to be sensitive to DEHP following pre-/postnatal exposure (Arcadi et al., 1998). A LOAEL of 3.5 mg/kg bw/day was identified for effects on the testes from this study. However, there are some limitations in this study due to dosing uncertainties and therefore it is not used for risk characterisation. Also testicular lesions, including Leydig cell hyperplasia and adenoma, have been observed in three-month old F1 male rats exposed to dibutyl phthalate *in utero* (gestation days 12-21: the prenatal period of male reproductive tract differentiation in the rat) (Mylchreest et al., 1999). Furthermore, young rats have been shown to be more sensitive to DEHP-induced testicular toxicity (Sjöberg 1985c, 1986b), however, this age-specific sensitivity is not fully characterised. In general, the foetus, is particularly sensitive to certain

toxicants during specific stages of early embryonic development and/or critical stages of organogenesis. Also exposure during the pre- and postnatal period up to puberty may cause effects which are not manifested until later in life e.g. endocrine disruption.

2) There is likely an exposure of infants through several different routes (see **Table 4.61** e.g. through baby food and breast milk), and instead of calculation of combined exposure, it is allowed to affect the setting of minMOS.

The MOS values are considered to clearly indicate either **conclusion (ii)** or **conclusion (iii)**.

Concerning the occurrence of DEHP in toys and child-care articles, it has been indicated by reputable toy manufacturers that DEHP is no longer recommended as a PVC plasticiser in toys and child-care articles. It is also indicated in market surveys by Greenpeace and others that there is a decreased trend in the use of DEHP in toys. However, it is well known that DEHP and some other phthalates are interchangeable in their use as PVC plasticisers. Also suppliers of PVC toys on the market seem not to be aware of precisely which phthalate is used as plasticiser. Furthermore, in cases when the toys are imported, first hand knowledge on phthalate content simply may not be available, since toy manufacturers may be unaware of this issue in other parts of the world. Because the results of the risk assessment clearly show concern for children exposed to DEHP used in plasticised PVC toys and child-care articles, a risk reduction strategy is required to ensure that the current and future uses of DEHP are appropriately controlled.

Summary and conclusion

There is concern with regard to testicular effects, fertility, and toxicity to kidneys, on repeated exposure as a consequence of oral exposure route to toys and child-care articles, and multiple routes of exposure. No risk is identified for exposure from car interiors. There is no concern for the acute toxicity, irritation and sensitising effects, carcinogenicity, and mutagenicity.

Conclusion (iii)

4.1.3.4 Medical equipment

4.1.3.4.1 Risk characterisation of patients

Exposure scenarios of concern in the risk characterisation of DEHP include those where patients in different sub-population (e.g. new-born/premature, children, adults and elderly) can be exposed to DEHP via medical equipment (for exposure scenarios; see **Table 4.10** and **Table 4.11**). Also a lifetime exposure scenarios have been considered. It should be noted that this risk assessment does not consider medical benefits.

MOSs presented here have been derived based on the following scenarios

1. Human internal exposure of adults *via* long term haemodialysis compared with NOAELs derived from animal oral studies for different critical endpoints (see **Table 4.67**)
2. Human internal exposure of adults *via* long term blood transfusion compared with NOAELs derived from animal oral studies for different critical endpoints (see **Table 4.67**)

3. Human internal exposure of children *via* long term blood transfusion compared with NOAELs derived from animal oral studies for different critical endpoints (see **Table 4.67**)

4. Human internal exposure of neonates *via* transfusion (no more details given) compared with NOAELs derived from animal oral studies for different critical endpoints. No information on exposure period was given but it is considered to be short term (see **Table 4.67**). However, the available short term toxicity studies in animals are not considered relevant for humans. Therefore, the NOAELs for effects on testes, fertility and development, but not long term studies, are used to derive MOS.

5-7. Lifetime human internal exposure via different infusions compared with NOAEL derived from long term animal oral studies for RDT (see **Table 4.68**).

Based on the available data it was not, however, possible to derive a quantitative MOS for exposure of premature babies which may be exposed via medical articles/equipment e.g. extracorporal oxygenation, feeding.

Table 4.67 Calculation of MOS for medical treated persons exposed to DEHP. Human internal exposure by i.v. routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
1. Long-term haemodialysis (adults)					
Intravenous					
RDT	3.1	29	14.5	4.6	(iii)
Testicular	3.1	4.8	4.8	2	(iii)
Fertility	3.1	20	10	3.2	(iii)
Developmental	3.1	4.8	4.8	2	(iii)
2. Long-term blood transfusion (adults) Haemophiliacs					
Intravenous					
RDT	0.03	29	14.5	483	(ii)
Testicular	0.03	4.8	4.8	160	(ii)
Fertility	0.03	20	10	333	(ii)
Developmental	0.03	4.8	4.8	160	(ii)
3. Long term blood transfusion (children)					
Intravenous					
RDT	0.075	29	14.5	193	(ii)
Testicular	0.075	4.8	4.8	64	(iii)
Fertility	0.075	20	10	133	(ii)

Table 4.67 continued overleaf

Table 4.67 continued Calculation of MOS for medical treated persons exposed to DEHP. Human internal exposure by i.v. routes compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
4. Transfusions (neonates)					
Intravenous					
Testicular	1.7	4.8	4.8	3	(iii)
Fertility	1.7	20	10	5.9	(iii)

1) Internal exposure based on measured data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular and developmental toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Table 4.68 Calculation of MOSs from lifetime exposure to DEHP during infusions. Human internal exposure by *i.v.* route compared with lifetime animal oral studies from RTD[2]; $NOAEL_{oral}$ = 29 mg/kg bw/day

Exposure scenario	Exposure[1] (mg/kg bw/d)	MOS_{NOAEL}[3]	$MOS_{NOAEL;CORR}$[4]	Conclusion
5. Infusion of platelets	0.001	29,000	14,500	(ii)
6. Haemodialysis	0.099	293	146	(ii)
7. Autopheresis	0.00058	50,000	25,000	(ii)

1) Internal exposure based on measured data (see Table 4.60)
2) 2-Year rat oral study
3) 100% bioavailability equivalence assumed
4) 50% bioavailability equivalence assumed

Factors considered when calculating MOS and or determining conclusion

Factors concerning various exposure scenarios are detailed under "Issues considered when calculating MOS and/or determining the conclusions"; Section 4.1.3.1. Additionally, some factors specific for exposure via medical equipment have also to be considered:

- DEHP is used widely in different medical equipment.
- Intravenous dosing can result in an extensive exposure which is 100% bioavailable.
- Patients exposed to DEHP are represented by different vulnerable subgroups including babies, children, and the elderly. The medical condition of patients (e.g. reduced kidney function) may render them more susceptible to DEHP-induced toxicity. For example, DEHP has been shown to have effects on the kidney and heart in repeated dose toxicity studies, and effects on cardiovascular system and lungs following a single iv administration. These effects can be of importance for patients undergoing dialysis and/or blood transfusion, and in extracorporal oxygenation in newborns.
- Young patients are considered to be a vulnerable subpopulation as a number of physiological, biochemical, genitical, and anatomical differences exist between children and adults (see "Risk characterisation of children consumers").
- During treatment with pharmaceuticals drug-interactions with DEHP may occur.

The resulting minMOS for all end-points is 100 in adults and (older) children. In neonates, it additionally needs to be considered that testicular toxicity is a serious end-point, especially in this very sensitive life-stage, and that additional exposure to DEHP occurs via, e.g., breast milk, warranting a minMOS of 250 (but the benefits of the treatment needs to be considered as well).

Summary and conclusion

Based on a quantitative risk assessment, there is concern for some or all end points: testicular, fertility, and developmental (adults only) for the exposure scenarios

- long term haemodialysis in adults
- long term blood transfusion in children
- transfusions in neonates

Calculating the MOS values on data from human lifetime exposure, there is no concern for the endpoint RTD (based on lifetime animal data) for haemodialysis, infusion of platelets, and autopheresis.

Based on a qualitative risk assessment, there is concern for all end points: testicular, fertility, RDT for extracorporal oxygenation in children

Conclusion (iii).

4.1.3.5 Humans exposed via the environment

The major contribution to regional exposure is from food products. For children this is primarily from diary products and for babies from infant formulae and breast milk. DEHP in food may originate from the environment and/or during food processing and/or from food packaging material. It is, however, not possible to distinguish between these sources.

The information used in EUSES is based on environmental monitoring and default data. Monitoring data for milk and air has been used for the regional estimation of exposure, monitoring data for milk also in all local scenarios. The concentration of 0.05 mg/l DEHP in milk (Petersen, 1991) and a concentration of 19 ng/m^3 in air are used (ECPI, 2000). MOS has been derived based on EUSES and separately on monitoring data for infant formulae (MAFF, 1998a,c), and breast milk (Gruber, 1998). Although the monitoring data on DEHP in milk is considered reliable, it may represent the contribution of DEHP from the environment and food contact materials (processing and packaging). Further information concerning the derivation of the exposure values and different scenarios is presented in Section 4.1.1.4. The total daily intake via air, drinking water and food at local scale and regional scale calculated by EUSES are summarised in **Table 4.13**, **Table 4.14** and **Table 4.15**.

Exposure scenarios considered important for adults and children, concern

Adults

- Exposure calculated from measured urinary excretion of DEHP-metabolites in humans (also covering children)
- Environmental exposure of adults estimated with EUSES (see **Table 4.69**)

Children

- Environmental exposure of children estimated with EUSES (see **Table 4.70** and see **Table 4.71**)

Babies

- Exposure from infant formulae based on monitoring data (see **Table 4.72** and see **Table 4.73**)
- Exposure from breast milk based on monitoring data (see **Table 4.74** and see **Table 4.75**)

However, consumption of mussels is not included in EUSES. Exposure to DEHP through the consumption of mussels may be of importance as the BCF for mussels is high (2,500) and certain subpopulations can consume large quantities of mussels.

Exposure of adults as calculated from urinary excretion of DEHP-metabolites

Based on analyses of DEHP-metabolites in urine of (non-occupationally exposed) Germans, the daily intake of DEHP was calculated by the authors to 52.1 μg/kg/day (95^{th} percentile), based on the secondary metabolites of MEHP in urine (Koch et al., 2003a). It should be noted that there are some uncertainties in this figure, as the calculation is based on the assumption that 25% of the ingested DEHP is excreted as urinary metabolites. The rapporteur believes that the approach is scientifically sound, and that the conversion factors are in conformity with the overall database, although in the lower end. The inclusion of secondary metabolites of MEHP in the analyses is also a clear advantage as it results in analyses of a larger share of the given substance and gives appropriate consideration of potential fast metabolisers of MEHP. However, a recent study using the best available analytical technique and deuterium-labelled DEHP (Koch et al., 2003d), has given conversion factors 3-fold higher than the ones originally used by Koch et al. (2003a). Although the new data is only based on one individual, the quality of this study warrants it's use. Based on this new data, the regional exposure is recalculated into 17 μg DEHP/kg/day (95^{th} percentile), which is the value used in the risk characterisation.

The studied German group (Koch et al 2003a) included some children, although the median age was 33 years of age. Other studies on urinary excretion of MEHP have suggested higher exposure of children than of adults (Brock et al., 2002, US CDC, 2003, Tsumura et al 2001b, Koch et al 2004), causing some uncertainty whether the exposure estimate chosen (17 ug/kg/day) may also be considered to represent the regional exposure of the whole population, including children.

It is generally believed that food is the major source of human DEHP-exposure, and recent Japanese data support that food can be contaminated with DEHP from different articles (e.g. PVC-gloves or tubings, as shown by Tsumura et al 2001b). Thus, this intake data has been chosen to represent exposure indirectly via the environment. The possible contamination of food from, e.g. PVC-items, is of course not included in the EUSES-model. Thus, the EUSES estimated human exposure (see **Table 4.15**) do not consider contamination of food from items, which is a likely reason as to why the measured levels (based on urinary excretion) are higher and probably more relevant than those estimated by EUSES. The chosen exposure estimate (17 μg/kg/day) can be compared with measured DEHP concentrations in food from Denmark and Japan, indicating intake levels up to 16 μg/kg/day and 59.9 μg/kg/day, respectively.

When comparing the regional exposure estimate of 17 μg/kg/day with the NOAELs, the results are as follows:

	NOAEL	MOS	Conclusion
RTD	14.5 mg/kg/day	852	(ii)
Testicular tox.	4.8 mg/kg/day	282	(ii)
Fertility	10 mg/kg/day	588	(ii)
Developmental tox.	4.8 mg/kg/day	282	(ii)

When determining the conclusions, MOS-values > 100 are considered to indicate no concern for adults and (older) children.

Summary/conclusions

Based on the results for regional exposure to DEHP of the whole population, **conclusion (ii)** applies for all end-points:

Conclusion (ii).Environmental exposure of adults estimated with EUSES

MOSs derived for adults are presented in **Table 4.69**.

Table 4.69 EUSES derived MOS values for adults and different exposure scenarios

Scenario	MOS Total and conclusions for different oral NOAELs							
	RDT		Testicular		Fertility		Development	
NOAEL[1]:	14.5[2]		4.8[3]		10[4]		4.8[5]	
Life cycle stage	MOS	Concl.	MOS	Concl.	MOS	Concl.	MOS	Concl.
Local:								
Production								
site 1.	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 2	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 3*	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 4	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 5	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 6	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 7*	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 8	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 9*	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 10.*	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 11.	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
site 12.	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)

Table 4.69 continued overleaf

Table 4.69 continued EUSES derived MOS values for adults and different exposure scenarios

Scenario	MOS Total and conclusions for different oral NOAELs							
	RDT		Testicular		Fertility		Development	
NOAEL[1]:	14.5[2]		4.8[3]		10[4]		4.8[5]	
Life cycle stage	MOS	Concl.	MOS	Concl.	MOS	Concl.	MOS	Concl.
2 Processing of polymer products								
2a Calendering	641	(ii)	212	(ii)	442	(ii)	212	(ii)
2b. Extrusion comp	> 1,000	(ii)	480	(ii)	997	(ii)	480	(ii)
2c. Extrusion prod	> 1,000	(ii)	480	(ii)	997	(ii)	480	(ii)
2d. Spread coating with air cleaning	> 1,000	(ii)	415	(ii)	865	(ii)	415	(ii)
2d. Spread coating without air cleaning	432	(ii)	143	(ii)	298	(ii)	143	(ii)
2e. Other plastisol with air cleaning	> 1,000	(ii)	685	(ii)	> 1,000	(ii)	685	(ii)
2e. Other plastisol without air cleaning	489	(ii)	162	(ii)	337	(ii)	162	(ii)
3 Non-polymer processing/ formulation								
3a Sealants/adhesives form.	463	(ii)	154	(ii)	320	(ii)	154	(ii)
3b Sealants/adhesives proc.	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
4a Lacquers and paint form.	559	(ii)	185	(ii)	385	(ii)	185	(ii)
4b Lacquers and paint proc.	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)
5a Prinitng ink formulation	555	(ii)	184	(ii)	383	(ii)	184	(ii)
6 Ceramic formulation	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1000	(ii)
7a. Municipal STP	> 1,000	(ii)	648	(ii)	> 1,000	(ii)	648	(ii)
8a Paper recycling	> 1,000	(ii)	596	(ii)	> 1,000	(ii)	596	(ii)
8b Waste car shredder	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1000	(ii)
8c Waste incineration	> 1,000	(ii)	> 1,000	(ii)	> 1,000	(ii)	> 1000	(ii)
Regional	**15,000**	**(ii)**	**4,970**	**(ii)**	**10,360**	**(ii)**	**4970**	**(ii)**

1) The oral bioavailabilty of DEHP is only 50% in adults. EUSES 1.0 cannot accout for this and therefore overestimates the internal exposure for adults with a factor of two. Instead of reducing the calculated daily intake for all scenarios by a factor of two the systemic NOAELs have been doubled in the EUSES modelling to compensate for this.
2) 2-Year rat oral study.
3) 3-generation oral reproductive toxicity study in rats.
4) Continuous breeding study in mice.
5) 3-generation oral reproductive toxicity study in rats

For local and regional exposure, **conclusion (ii)** is reached for adults.

Factors considered when calculating MOS and or determining conclusion:

Factors concerning various exposure scenarios are detailed under "Issues considered when calculating MOS and/or determining the conclusions" (see Section 4.1.3.1).

Concerning different toxicological end-points, different cut-offs are selected when the derived MOS values do not clearly indicate a **conclusion (ii)** or **conclusion (iii)**, or clarification is required:

- A MOS > 100 is considered an acceptable cut-off for effects on kidney (RDT) because the study is long-term (two-years).
- A MOS of > 100 is considered an acceptable cut-off for effects on testes. A lower cut-off compared with children is considered acceptable since adult rats are less sensitive than young animals (Wolfe et al., 2003; Poon et al., 1997). Also a study with marmosets (Kurata et al., 1998) indicates that they are less sensitive to DEHP than mature rats, mice, hamsters and ferrets, and young rats (e.g. Sjöberg et al., 1985c). The sensitivity of marmosets to MEHP has not been tested.
- A MOS of > 100 is considered an acceptable cut-off for effects on fertility. Rats have been shown to be sensitive to DEHP following pre-/postnatal exposure (Arcadi et al., 1998). A LOAEL of 3.5 mg/kg bw/day was identified for effects on testes from this study. Also testicular lesions, including Leydig cell hyperplasia and adenoma, have been observed in three-month old F1 male rats exposed to dibutyl phthalate *in utero* (gestation days 12-21: the prenatal period of male reproductive tract differentiation in the rat) (Mylchreest et al., 1999). In general, the foetus is particularly sensitive to certain toxicants during specific stages of early embryonic development and/or critical stages of organogenesis. Also exposure during the pre- and postnatal period (up to puberty) may cause effects, which are not manifested until later in life (e.g., endocrine disruption).
- A MOS of > 100 for developmental toxicity is considered to be an acceptable cut-off.

Summary/conclusion

- For adults there is no concern for any of the toxicological endpoints studied from regional or local exposure as estimated by EUSES.

Based on the EUSES-results for regional and local exposure from food, water and air assessment for adults, **conclusion (ii)** applies.

Environmental exposure of children determined by EUSES

Assumptions made were that the children's body weight was 8 kg bw, daily intake of diary products 1.68 kg/day milk, and inhalation rate = 9.3 m^3/day (children 0.5-3 year, respiratory volume while at rest 168 l/hour, activity factor 2.3 -moderate activity (Whalan et al., 1997).

MOSs derived for children for generic sites are presented in **Table 4.70**, and MOSs derived for children modelled using reported site-specific emission data are presented in **Table 4.71**.

Table 4.70 EUSES derived MOS values for children and different generic (except production 1-12) exposure scenarios

Scenario	MOS Total and conclusions for different oral NOAEL/LOAELs					
	RDT[1]		Testicular		Fertility	
NOAEL:	14.5		4.8[2]		10[3]	
Life cycle stage	MOS	Concl.	MOS	Concl.	MOS	Concl.
Local:						
1 Production						
site 1.	747	(ii)	247	(ii)	515	(ii)
site 2	522	(ii)	172	(ii)	360	(ii)
site 3	747	(ii)	247	(ii)	515	(ii)
site 4	743	(ii)	246	(ii)	512	(ii)
site 5	377	(ii)	125	(ii)	260	(ii)
site 6	278	(ii)	92	(iii)	191	(ii)
site 7.	747	(ii)	247	(ii)	515	(ii)
site 8	659	(ii)	218	(ii)	454	(ii)
site 9	747	(ii)	247	(ii)	515	(ii)
site 10.	747	(ii)	247	(ii)	515	(ii)
site 11.	379	(ii)	126	(ii)	261	(ii)
site 12.	357	(ii)	118	(ii)	246	(ii)
2 Processing of polymer products						
2a Calendering	68	(iii)	22	(iii)	47	(iii)
2b. Extrusion comp	138	(ii)	46	(iii)	95	(iii)
2c. Extrusion prod	138	(ii)	46	(iii)	95	(iii)
2d. Spread coating, with air cleaning	123	(iii)	41	(iii)	85	(ii)
2d. Spread coating, without air cleaning	46	(iii)	15	(iii)	32	(iii)
2e. Other plastisol, with air cleaning	188	(ii)	62	(iii)	130	(ii)
2e. Other plastisol, without air cleaning	52	(iii)	17	(iii)	36	(iii)

Table 4.70 continued overleaf

Table 4.70 continued EUSES derived MOS values for children and different generic (except production 1-12) exposure scenarios

Scenario	MOS Total and conclusions for different oral NOAEL/LOAELs					
	RDT		Testicular		Fertility	
NOAEL:	14.5[1]		4.8[2]		10[3]	
Life cycle stage	**MOS**	**Concl.**	**MOS**	**Concl.**	**MOS**	**Concl.**
3 Non-polymer processing/formulation						
3a Sealants/adhesives form.	50	(iii)	16	(iii)	34	(iii)
3b Sealants/adhesives proc.	743	(ii)	246	(ii)	512	(ii)
4a Lacquers and paint form.	59	(iii)	20	(iii)	41	(iii)
4b Lacquers and paint proc.	345	(ii)	114	(ii)	238	(ii)
5a Printing ink formulation	59	(iii)	20	(iii)	41	(iii)
6 Ceramic formulation	453	(ii)	150	(ii)	312	(ii)
7a. Municipal STP	174	(ii)	58	(iii)	120	(ii)
8a Paper recycling	167	(ii)	55	(iii)	115	(ii)
8b Waste car shredder	747	(ii)	247	(ii)	515	(ii)
8c Waste incineration	703	(ii)	233	(ii)	485	(ii)
Regional	**747**	**(ii)**	**247**	**(ii)**	**515**	**(ii)**

* For scenarios 3, 7, 9, 10 and 8b EUSES calculates MOS-values that are higher than the regional MOS. These MOS values ares overwritten with the regional MOS.

1) 2-Year rat oral study.
2) 3-generation oral reproductive toxicity study.
3) Continuous breeding study.

Table 4.71 MOS values for children and different exposure scenarios based on reported site-specific data

Scenario	MOS Total and conclusions for different oral NOAEL/LOAELs					
	RDT		Testicular		Fertility	
NOAEL:	14.5[1]		4.8[2]		10[3]	
Life cycle stage	MOS	Concl.	MOS	Concl.	MOS	Concl.
Local:						
2a Calendering						
site F3	622	(ii)	206	(ii)	429	(ii)
site S4	426	(ii)	141	(ii)	294	(ii)
site S6	462	(ii)	153	(ii)	319	(ii)
2b Extrusion compound						
site S5	776	(ii)	257	(ii)	535	(ii)
2c Extrusion product						
site F2	508	(ii)	168	(ii)	350	(ii)
site F7	649	(ii)	215	(ii)	448	(ii)
site I9	613	(ii)	203	(ii)	423	(ii)
site S11	776	(ii)	257	(ii)	535	(ii)
2d Spread coating						
site F1	580	(ii)	192	(ii)	400	(ii)
site S8	326	(ii)	108	(ii)	225	(ii)
2? Polymer, not known						
site ES12	622	(ii)	206	(ii)	429	(ii)
site ES13	710	(ii)	235	(ii)	490	(ii)
3a Sealants/adhesives form						
adhesives site F10	562	(ii)	186	(ii)	388	(ii)

1) 2-Year rat oral study.
2) 3-generation oral reproductive toxicity study.
3) Continuous breeding study.

There is no concern for regional exposure of children.

For local exposure based on generic data, **conclusion (iii)** is reached for children in many scenarios. However, when modelling local exposure based on the site-specific data that has been received from some plants, there is no concern. However, site-specific data is limited to a few out of many sites. The volume used by these sites covers only a few percent of the total volume used in these use areas. The data can therefore not be taken as representative for all other sites (see also section 3.1.1.2.1). As a realistic worst case it has been assumed that there are sites having releases as large as the modelled generic ones.

Factors considered when calculating MOS and or determining conclusion

Factors common for all exposure scenarios are presented under "Issues considered when calculating MOS and/or determining the conclusions" (see Section 4.1.3.1).

Concerning human exposure, differences in oral and inhalation uptake between children and adults has been accounted for as the internal exposures has been estimated based on different bioavailability factors for children in comparison with adults (GI-tract: 100% in comparison with 50%; and, inhalation: 100% in comparison with 75%). Dermal exposure is not considered for this population.

Thus, for all three end-points (repeated dose nephrotoxicity, testicular toxicity, and fertility), a minMOS of 100 is used. In setting the minMOS, it is also considered that, on the one hand the modelling is made for infants, and on the other, that the food basket quantity is very conservatively chosen.

Summary/conclusion

- For children there is concern with regard to testicular effects, fertility and toxicity to kidneys, on repeated exposure resulting from several generic local exposure scenarios, but not when site-specific release data has been used.

Based on the results for local exposure from food, water and air assessment for children **conclusion (iii)** applies.

Risk characterisation for babies (Infant formulae)

MOSs derived for babies are presented in **Table 4.72** and **Table 4.73**.

Table 4.72 Calculation of MOS for newborn babies (0-3 months old) exposed to DEHP via infant formulae. Human internal exposure by oral route compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
RDT	0.013	29	14.5	1,115	(ii)
Testicular	0.013	4.8	4.8	369	(ii)
Fertility	0.013	20	10	769	(ii)

1) Internal exposure based on measured data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAEL for testicular toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Table 4.73 Calculation of MOS for infants (6+ months old) exposed to DEHP via infant formulae. Human internal exposure by oral route compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
RDT	0.008	29	14.5	1,812	(ii)
Testicular	0.008	4.8	4.8	600	(ii)
Fertility	0.008	20	10	1,250	(ii)

1) Internal exposure based on measured data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Factors considered when calculating MOS and or determining conclusion

Factors common for all exposure scenarios are presented under "Issues considered when calculating MOS and/or determining the conclusions" (see Section 4.1.3.1).

Concerning human exposure, differences in oral uptake between children and adults has been accounted for as the internal exposures has been estimated based on different bioavailability factors for children in comparison with adults (GI-tract: 100% in comparison with 50%).

Concerning different toxicological end-points, different cut-offs for children are selected when the derived MOS values do not clearly indicate a **conclusion (ii)** or **conclusion (iii)**, or clarification is required:

- A MOS >100 is considered an acceptable cut-off for effects on kidney (RDT) because the study is long-term (two-years).
- A MOS of around 250 for testicular effects and fertility is considered as acceptable cut-off for new-borns (0-3 months of age) because: 1) Testicular toxicity is a serious end-point, especially in this very sensitive (developing) life-stage. Rats have been shown to be sensitive to DEHP following pre-/postnatal exposure (Arcadi et al., 1998). A LOAEL of 3.5 mg/kg bw/day was identified for effects on the testes from this study. A NOEAL of 3.7 mg/kg/day (based on sertoli cell vacuolisation at 37 mg/kg/day) was found in a 90-days study with exposure starting when the rats were relatively young (Poon et al., 1997). Also testicular lesions, including Leydig cell hyperplasia and adenoma, have been observed in three-month old F1 male rats exposed to dibutyl phthalate *in utero* (gestation days 12-21: the prenatal period of male reproductive tract differentiation in the rat) (Mylchreest et al., 1999) indicating a potential for interaction between different phthalates with regard to testicular toxicity. Furthermore, young rats have been shown to be more sensitive to DEHP-induced testicular toxicity (Sjöberg 1985c, 1986b), which is also evident in the 3-generation study (Wolfe et al., 2003). In general, the foetus, is particularly sensitive to certain toxicants during specific stages of early embryonic development and/or critical stages of organogenesis. Also exposure during the pre- and postnatal period up to puberty may cause effects that are not manifested until later in life e.g. endocrine disruption. With two multi-generation studies at hand (Wolfe at al., 2003; Schilling et al., 2001), the uncertainty with regard to the effect level has decreased substantially. Infants at the age of 3-12 months of age have likely passed the most vulnerable developmental period and a slightly lower minMOS (than for newborns) can be used for this group. However, infants from 3 months of age will also be exposed to DEHP from other sources as estimated in **Table 4.70**. However, the relative contribution from infant formula, solid foods and breast milk will greatly vary for different subgroups within this population depending on the child's individual habits and also on cultural practices associated with different regions and countries. Therefore, it is considered that there is a large degree of uncertainty attached with the lowering of the minimal MOS below 200 for this age group and population as a whole. 2) There is exposure of infants through several different routes (**Table 4.61** Combined exposure, e.g., through baby food, indoor air, indoor dust, toys and other soft PVC), and instead of calculation combined exposure, it is allowed to affect the setting of minMOS. Examples of worst-case exposure levels through these routes are; < 22 μg/kg/day via indoor air, 59.9 μg/kg/day via one brand of Japanese baby food (Tsumura et al. 2001b), < 200 μg/kg/day via mouthing on soft PVC. In addition, median concentrations of 195 μg DEHP/g dust has been reported from the UK, likely contributing to the internal exposure (Santillo et al. 2003).

Summary/conclusion

- For new-borns and infants there is no concern from the consumption of infant formulae for any effects.

Based on the results for infants exposed via infant formulae **conclusion (ii)** applies.

Exposure from breast milk based on monitoring data

MOSs derived for children are presented in **Table 4.74** and **Table 4.75**.

Table 4.74 Calculation of MOS for new-borns 0-3 months old, exposed to DEHP via breast milk. Human internal exposure by oral route compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
RDT	0.006	29	14.5	2,339	(ii)
Testicular	0.006	4.8	4.8	774	(ii)
Fertility	0.006	20	10	1,613	(ii)

1) Internal exposure based on measured data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Table 4.75 Calculation of MOS for infants 3-12 months old, exposed to DEHP via breast milk. Human internal exposure by oral route compared with animal oral studies

Effect	Exposure[1] (mg/kg bw/d)	NOAEL[2] (mg/kg bw/d)	$NOAEL_{Systemic}$[3] (mg/kg bw/d)	MOS ($_{NOAEL, Systemic}$)[4]	Conclusion
RDT	0.002	29	14.5	6,042	(ii)
Testicular	0.002	4.8	4.8	2,000	(ii)
Fertility	0.002	20	10	4,167	(ii)

1) Internal exposure based on measured data (see Table 4.60)
2) NOAEL derived from oral studies in rats (see Table 4.63)
3) Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
4) MOS derived based on the systemic oral NOAEL for rats

Factors considered when calculating MOS and or determining conclusion

Factors common for all exposure scenarios are presented under "Issues considered when calculating MOS and/or determining the conclusions" (see Section 4.1.3.1).

Concerning human exposure, differences in oral and inhalation uptake between children and adults has been accounted for as the internal exposures has been estimated based on different bioavailability factors for children in comparison with adults (GI-tract: 100% in comparison with 50%).

Concerning different toxicological end-points, different cut-offs for children are selected when the derived MOS values do not clearly indicate a **conclusion (ii)** or **conclusion (iii)** , or clarification is required:

- A MOS > 100 is considered an acceptable cut-off for effects on kidney (RDT) because the study is long-term (two-years).
- A MOS of around 250 for testicular effects and fertility is considered as acceptable cut-off for new-borns (0-3 months) because: 1) Testicular toxicity is a serious end-point, especially in this very sensitive (developing) life-stage. Rats have been shown to be sensitive to DEHP following pre-/postnatal exposure (Arcadi et al., 1998). A LOAEL of 3.5 mg/kg bw/day was identified for effects on the testes from this study. However, there are some limitations in this study due to dosing uncertainties and therefore it is not used for risk characterisation. A NOEAL of 3.7 mg/kg/day (based on sertoli cell vacuolisation at 37 mg/kg/day) was found in a 90-days study with exposure starting when the rats were relatively young (Poon et al., 1997). Also testicular lesions, including Leydig cell hyperplasia and adenoma, have been observed in three-month old F1 male rats exposed to dibutyl phthalate *in utero* (gestation days 12-21: the prenatal period of male reproductive tract differentiation in the rat) (Mylchreest et al., 1999), indicating a potential for interaction between different phthalates with regard to testicular toxicity. Furthermore, young rats have been shown to be more sensitive to DEHP-induced testicular toxicity (Sjöberg 1985c, 1986b), which is also evident in the 3-generation study (Wolfe et al., 2003). In general, the foetus, is particularly sensitive to certain toxicants during specific stages of early embryonic development and/or critical stages of organogenesis. Also exposure during the pre- and postnatal period up to puberty may cause effects, which are not manifested until later in life e.g. endocrine disruption. However, with two multi-generation studies at hand (Wolfe at al., 2003; Schilling et al., 2001), the uncertainty with regard to the effect level has decreased substantially. Infants at the age of 3-12 months of age have likely passed the most vulnerable developmental period and a slightly lower minMOS (than for newborns) can be used for this group. However, infants from 3 months of age will also be exposed to DEHP from other sources as estimated in **Table 4.70**. The relative contribution from breast milk, solid foods and infant formula will greatly vary for different subgroups within this population depending on the child's individual habits and also on cultural practices associated with different regions and countries. Therefore, it is considered that there is a large degree of uncertainty attached with the lowering of the minimal MOS below 200 for this age group and population as a whole.

  2) There is exposure of infants through several different routes (see **Table 4.61**, e.g., through baby food, indoor air, indoor dust, toys and other soft PVC), and instead of calculating combined exposure, it is allowed to affect the setting of minMOS. Examples of worst-case exposure levels through these routes are; < 22 µg/kg/day via indoor air, 59.9 µg/kg/day via one brand of Japanese baby food (Tsumura et al., 2001b), < 200 µg/kg/day via mouthing on soft PVC. In addition, median concentrations of 195 µg DEHP/g dust has been reported from the UK, likely contributing to the internal exposure (Santillo et al. 2003). However, overall the breast milk is likely the biggest source of exposure. Barr et al. (2003), Brock et al. (2002) and Koch et al. (2004) have also shown that the urinary concentrations of MEHP is higher in children than in adults, which suggests that the exposure to DEHP is higher in children than in adults.

Conclusion

For new-borns and infants there is no concern from the consumption of breast milk for any effect.

Conclusion (ii).

4.1.3.6 Combined exposure

Several separate and combined exposure groups can be identified. Quantitative results for combined populations have not been derived. An overall examination of the available data indicates that adults, children and babies are potentially exposed to DEHP from several different sources. These exposures may be by different exposure routes and occur either sequentially or separately. The exposures may also vary in frequency occurring at different ages, and for different durations. It is apparent that exposure to DEHP occurs for a life-time but the amount, frequency and source change. It is generally difficult to quantitatively assess combined exposure, as addition of several reasonable worst-case values (e.g. 95th percentile exposure values) could lead to a rather unrealistic sum, because it is perhaps not that likely that an individual belongs to the 5% most highly exposed individuals for all different exposure routes/sources. However, this possible shortcoming has been overcome in adults by basing measurements of exposure on urinary excretion of DEHP metabolites. The combined exposure is therefore considered specifically in setting the minMOS for the different exposure scenarios for babies, which is believed to be the most sensitive sub-population and for which several different exposure routes/sources exist (breast milk, infant formula, baby food, mouthing on soft PVC, indoor air, and by dermal and oral exposure via indoor dust containing DEHP).

Summary/conclusion

Characterisation of combined exposure may indicate reason for concern for new-borns. As this potential will be addressed in the risk assessment for new-borns in the section "Human exposed via the environment", no conclusion will be drawn in this specific section.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Flammability, explosive and oxidising properties are not considered to form a hazard hence further characterisation is not undertaken in this report. In addition, there is no need for further information and/or testing with regard to physico-chemical properties. Therefore it is considered that **conclusion (ii)** applies.

5 RESULTS

5.1 INTRODUCTION

DEHP is widely used in many products in the society. The main use of DEHP is as a plasticizer in polymer products, mainly in flexible PVC. DEHP occurs in many different life-cycle stages indicating both point and multiple sources of exposure. The main stages identified include:

- Production of DEHP
- Formulation of polymers
- Processing of polymers
- Formulation of non-polymers
- Processing of non-polymers
- End-use of products (articles) containing DEHP
- Waste management including:
  - Paper recycling
  - Car shredding
  - Incineration of DEHP containing products
  - Disposal on land fills of DEHP containing products
  - "Waste remaining in the environment"

Information is generally available for production of DEHP and processing of flexible PVC but to a lesser extent for formulation and processing of non-PVC and non-polymer uses.

5.2 ENVIRONMENT

5.2.1 General

The results summarised here are presented in detail in Section 3.

Emissions

DEHP is known to migrate slowly from polymer products during their entire lifetime. The total annual emission for DEHP (1997) is estimated to be about 29,000 tpa (see **Table 3.37**).

Depending on the environmental conditions emitted DEHP will evaporate, precipitate, biodegrade or be adsorbed to organic matter. The exposure analysis indicates that the main part of released DEHP originates from use and disposal of polymer products. This release is dispersed widely. Releases from industrial point sources are expected to cause local exposure.

The following contributions from different life-cycle stages is estimated:

Source	Emission contribution	Uncertainty in the estimate	Emission type
Production of DEHP	~2.5%	low	point sources
Industrial uses	~2.5%	medium	point sources
End-product uses	~32%	medium	wide disperse (+ point sources)
Waste handling*	~63%	high	wide disperse (+ point sources)

* Car shredding, Incineration + Land fills + Waste remaining in the environment

Considerable amounts of DEHP are introduced annually into the technosphere. The main use in persistent polymers causes long lifetimes and accumulation of DEHP in the society (dump sites included).

Some sources of emission are expected to have very long lifetimes (e.g. soil buried cables, waste remaining in the environment or building materials). This may cause increasing environmental exposures in the future. Since this type of emission seems to dominate for DEHP the interpretation of calculated regional PEC in relation to monitored data is difficult. However, DEHP has had a relatively stable consumption level in Europe the last 30 years. There are only a few products with service lives longer than that (e.g. soil buried cables). Therefore a major increase in concentration of DEHP in the environment is not to be expected over the coming years if the consumption volume and use pattern stay stable.

Environmental fate

DEHP enters the environment mainly via direct release to the air, release from sewage sludge and from solid waste. In the air, DEHP may occur both in vapour phase and as solid particles. The nature of these particles can be either aggregates of pure DEHP, polymer particles containing DEHP or particles of other material where DEHP is adsorbed to the surface. In sewage treatment plants "free" DEHP will be strongly adsorbed to the sludge. Particles formed by weathering of polymer products ("waste remaining in the environment") probably represent an important route of DEHP distribution. Since DEHP still remains in the polymer this distribution route results in a prolonged release period compared to non-polymer materials. Depending on the particle size, some of this waste can be distributed to the air compartment, while larger particles are distributed to the soil and sediment compartments.

DEHP is persistent to hydrolysis. However, evaporated DEHP will to some extent degrade in the atmosphere. Photodegradation is assumed to be of little importance in water and soil. The biodegradation of DEHP is varying in available studies. Under optimal conditions DEHP may be degraded rather fast. Microbial adaptation will obviously increase the degradation. Based on standard test method DEHP can be classified as readily biodegradable. However, under more realistic environmental conditions (i.e. DEHP adsorbed to organic matter) the degradation seems to slow down. In the final recipient, aquatic sediments, the anaerobic conditions reduce the degradation rate even further. The fate of DEHP in agricultural soil is unclear. A moderate-low biodegradation can be expected.

The biodegradation in sewage treatment plants is slow, probably due to strong adsorption of DEHP to organic matter. DEHP concentrations in sludge from municipal sewage treatment plants can therefore be rather high, although the biodegradation is strongly dependent on

environmental conditions. In aerobic conditions and high exposure (adapted organisms), DEHP is readily degraded. However, in anaerobic conditions, DEHP is persistent to biodegradation.

The degradation product MEHP (phthalic acid monoester) is formed during biodegradation. MEHP causes reproductive toxic effects in studies on mammals. Other ecotoxicological properties of MEHP are unknown.

The extent of formation of MEHP in the environment is unknown; however, the large amount of DEHP accumulated in the technosphere indicates a considerable potential for formation and distribution of MEHP. The fate of this metabolite can be expected to differ from DEHP's fate, due to different chemical/physical properties. It is not possible based on available data to estimate the environmental and human risks of MEHP formed in the environment.

The degree of contamination observed when analysing DEHP in environmental samples indicate a high diffuse contamination.

DEHP is found to bioaccumulate in aquatic organisms (BCF = < 2,700). The highest BCF values are observed in invertebrates. Adsorption of DEHP to particulate matter and organic surfaces enhances the BCF for filtraters and species with a high surface to body weight ratio. The lower values observed in vertebrates such as fish (BCF = 840), may also in part be due to a more effective metabolisation rate.

Effects

There are no reliable short term or long-term studies below the “apparent” water solubility of DEHP indicating effects on aquatic organisms exposed to DEHP via the water only. Hence a PNEC cannot be specified. However, effects have been shown on fish exposed to DEHP via food only. Therefore a $PNEC_{food}$ for fish of 16 mg/kg fresh food has been determined.

An overall PNEC of > 100 mg/kg dwt for sediment organisms, mainly based on a study on amphibian (frogs) embryos/larvae, has been chosen for this risk assessment.

Only one study was considered valid to be used for the risk assessment of micro-organisms in STP. No effects were observed and a $PNEC_{STP}$ > 200mg/l is used in the risk characterisation.

The PNEC for soil organisms is > 13 mg/kg dwt due to absence of effects on germination and growth of plants at the highest test concentration.

The PNEC of 3.3 mg/kg for predators has been calculated based on studies showing testicular damage in rats and mice.

5.2.2 Aquatic compartment (incl. sediment)

5.2.2.1 Micro-organisms in sewage treatment plants

The PEC/PNEC ratios are below one for all scenarios.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.2.2 Surface waters

Due to lack of effects at or below the "apparent" water solubility no PNEC can be specified. The conclusion is that there is no concern for aquatic species exposed via the water phase.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.2.3 Sediment dwelling organisms

Due its lipohilic nature and slow degradation under anaerobic conditions DEHP is often found in high concentrations in sediment. The PNEC is derived from a study where no effects were seen at the highest tested concentration and the other sediment toxicity studies indicates even lower sensitivity. Therefore the actual PNEC may be higher. Repeating the tests at higher test substance concentrations than those used is not proposed because data is already available for DEHP and because of the difficulties associated with testing very high concentrations of a substance. Further emission information could be requested to refine the exposure assessment, but since the same scenarios that have PEC/PNEC ratios > 1 for sediment dwelling organisms, also have PEC/PNEC ratios > 1 for the food chains based on aquatic organisms, the risk reduction strategy will have to address the emissions in these scenarios anyway. Further studies are therefore not requested at this point. Therefore a **conclusion (i)** "on hold" is reached.

Conclusion (i) There is a need for further information and/or testing.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for the other environmental spheres will eliminate the need for further information on sediment dwelling organisms.

This conclusion applies to the processing of polymers containing DEHP and for formulation of lacquers, paints, printing inks, sealants and/or adhesives containing DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data. The production sites with PEC/PNEC ratios above 1 have all ceased production.

5.2.3 Atmosphere

There are no data indicating risk for the atmosphere compartment.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.4 Terrestrial compartment

5.2.4.1 Soil organisms

The PNEC is based on a > NOEC value, thus the actual PNEC may be higher. Repeating the tests at higher test substance concentrations than those used is not proposed because data is already available for DEHP and because of the difficulties associated with testing very high concentrations of a substance. Further emission information could be requested to refine the

exposure assessment, but since the same scenarios that have PEC/PNEC ratios > 1 for soil organisms, also have PEC/PNEC ratios > 1 for the food chains based on terrestrial organisms, the risk reduction strategy will have to address the emissions in these scenarios anyway. Further studies are therefore not requested at this point. Therefore a conclusion (i) "on hold" is reached.

Conclusion (i) There is a need for further information and/or testing.

Further refinement of the assessment may remove some concern. However implementation of risk management measures to address the risks identified for the other environmental spheres will eliminate the need for further information on soil organisms.

This conclusion applies to the processing of polymers containing DEHP and for formulation of printing inks, sealants and/or adhesives containing DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

5.2.5 Secondary poisoning

The $PNEC_{oral}$ for DEHP in fresh food is 3.3 mg/kg for mammalians, 17 mg/kg for birds, and 16 mg/kg for fish (see Sections 3.2.4.2 and 3.2.1.6). In this risk assessment we propose that mammalians eat fish, birds eat mussels, and fish eat invertebrates in food chains based on aquatic exposure of DEHP. In the terrestrial food chain mammalians are supposed to eat DEHP exposed earthworms.

Food chains based on aquatic organisms

The PEC/PNEC ratios are below 1 for mammalians eating fish, and for fish eating invertebrates for all scenarios. For birds eating mussels the ratio is above 1 for 6 scenarios, two of which are production sites that have now ceased production. The generic, but not the site-specific, local risk characterisation for plastisol spread coating without air cleaning and printing ink, and sealants/adhesives formulation gave PEC/PNEC ratios > 1. Therefore a **conclusion (iii)** is reached.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the processing of polymers containing DEHP and for formulation of printing ink, sealants and/or adhesives containing DEHP. The scenarios that give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

Food chains based on terrestrial organisms

For mammalians eating earthworms the PEC/PNEC ratios are above 1 for the generic scenarios Plastisol spread coating without air cleaning, Other plastisol without air cleaning, Sealant and adhesives formulation and lacquers, paints and printing ink formulation. For all site-specific scenarios and all other local and regional scenarios the ratio is below 1.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the processing of polymers containing DEHP and for formulation of lacquers, paints, printing inks, sealants and/or adhesives, containing DEHP. The scenarios that

give concern are generic scenarios based on default emission data. There is no concern for the limited number of sites that have reported measured emission data.

5.3 HUMAN HEALTH

The results summarised here are presented in detail in Section 4.

The toxicity to exposure ratio for different human populations and scenarios has been used to derive the MOS. The lowest and most reliable NOAELs established in oral studies in animals have been used. These effects concern: repeated dose toxicity effects on kidney and testes, as well as effects on fertility and development. To correct for route-to-route extrapolation, systemic oral NOAELs for kidney and for fertility have been derived from oral NOAELs in rats: this is based on 50% oral bioavailability in adults. This extrapolation has not been necessary for the other end-points, as they are obtained from life-time studies covering phases with different absorption rates (50-100%).

Human populations exposed to DEHP are: workers, consumers including patients and indirect exposure to man via the environment. Also children and babies have been considered in this risk assessment. MOSs have been calculated for single and multiple pathways of exposure. For combined population, subpopulation combinations have been identified and exposure described in qualitative terms, hence, the total body burden has not been estimated. It should be noted that several exposure scenarios have been identified for different human populations. However, there may be other relevant exposure scenarios which have not been identified.

5.3.1 Human health (toxicity)

5.3.1.1 Occupational assessment

Three occupational exposure scenarios have been considered concerning exposure during production of DEHP, industrial use of DEHP and industrial end-use of products containing DEHP.

Worst-case exposure is assumed for the scenarios on production and industrial use, by using monitored data for inhalation exposure and modelled values for dermal exposure.

For the scenario of industrial end-use of products containing DEHP, it is assumed that relatively high work temperatures, aerosol generation and considerable skin contact occur. There is few quantitative and qualitative information available on technical control measures and personal protective equipment used during production and processing to establish their efficiency. There is concern for the testicular effects, fertility, toxicity to kidneys, on repeated exposure, and developmental toxicity for workers as a consequence of inhalation and dermal exposure. There is no concern for the acute toxicity, irritation and sensitising effects, carcinogenicity, and mutagenicity.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

5.3.1.2 Consumer assessment

Exposure scenarios considered important for adult consumers concern exposure from indoor air, PVC gloves and car interiors. The information used is based on measurements and modelling of DEHP in indoor air, absorption from gloves and car interiors.

Exposure scenarios considered important for children consumers concern exposure from toys and baby equipment, from indoor air and from car interiors. The information used is based on measurements of DEHP in artificial and human saliva for toys and baby equipment and modelled data for indoor air.

The results of the consumer assessment for adults is that **conclusion (ii)** applies:

There is no concern for exposure from indoor-air, gloves, car interiors and multiple pathways of exposure for all endpoints studied.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

The results of the consumer assessment for children is that **conclusion (iii)** applies:

There is concern for testicular effects, fertility and toxicity to kidneys, on repeted exposure, as a consequence of oral exposure route to toys and child-care articles, and multiple routes of exposure. No risk is identified for exposure from indoor air or car interiors. There is no concern for the acute toxicity, irritation and sensitising effects, carcinogenicity, and mutagenicity.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Concerning exposure of consumers from medical equipment, there is concern for some or all end points: testicular effects, fertility, toxicity to kidneys, on repeated exposure and developmental (excluding children) for the exposure scenarios

- long term haemodialysis in adults (testicular, fertility, toxicity to kidneys and developmental)
- long term blood transfusion in children (testicular)
- transfusions in neonates (testicular and fertility)

Calculating the MOS values on data from human lifetime exposure from medical equipment during infusions, there is no concern for the endpoint toxicity on repeated exposure (based on lifetime animal data) for haemodialysis, infusion of platelets and autopheresis.

Based on a qualitative risk assessment, there is concern for all end points: testicular effects, fertility, toxicity to kidneys, on repeated exposure for extracorporal oxygenation in children

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

5.3.1.3 Humans exposed via the environment

Exposure scenarios considered important for adults and children are:

- Environmental exposure of adults

- Environmental exposure of children
- Exposure of babies/infants from infant formulae
- Exposure of babies from breast milk

Environmental exposure has been estimated by two different approaches; by calculation of daily 'regional' intake of DEHP based on the measured excretion of DEHP-metabolites in a general population, and by the EUSES model. Using EUSES, worst-case exposure has been estimated for adults and children. Both environmental monitoring and default values were used in the model. This information was compared with NOAELs derived from oral studies. MOSs for both local and regional exposure have been estimated. The assessment based on measured excretion of DEHP-metabolites is believed to be more reliable (and covering potential contamination of food during handling and processing) than the one based on EUSES, and when it comes to regional exposure, only the conclusion from the former assessment (based on biomonitoring) is presented here. However, the assessment of local exposure is solely based on EUSES.

The result for man exposed indirectly via the environment is **conclusion (ii)** for regional exposure of adults.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

In the local exposure scenarios, there is concern for children for testicular, fertility and RDT effects in several of the local scenarios, but there is no concern for the adults. The scenarios that give concern for children are generic scenarios based on default values for children living in the vicinity of sites: processing polymer products; producing sealants/adhesives, lacquers and paints, or printing ink; municipal STP; and recycling paper. For municipal STP and paper recycling the only concern is for testicular effects. There is no concern for the limited number of sites that have reported emission data. Based on the results for local exposure from food, water and air assessment for children **conclusion (iii)** applies:

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

For exposure of new-born and infants via infant formulae and breast milk, monitoring data have been used. There is no concern (**conclusion (ii)**) for any end-point for new-borns and infants exposed via infant formulae or breast milk.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.3.1.4 Combined exposure

Exposure to DEHP apparently occurs during the entire human life time, from different sources. Exposure may therefore be equated with persistent low dose exposure. New-borns are probably the most sensitive sub-population, exposed via many sources, and perhaps at higher levels than the adults. However, combined exposure is considered in setting conclusions for the most sensitive sub-population, i.e. the new-borns, in the section "Humans exposed via the environment", and no conclusion will therefore be drawn specifically in this section on combined exposure.

5.3.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

6 REFERENCES

ABC-Laboratories (1991). Columbia, USA, ABC Final Report No.38811, 24.071991.

Adams WJ (1978) Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna. Monsanto Industrial Company report ES-SS-78-9.

Adams WJ and Calvert C (1983) Acute toxicity of di-(2-ethylhexyl) phthalate to Chironomus tentans. Monsanto Company report ES-82-SS-80

Adams WJ and Heidolph BB (1985) Short-cut chronic toxicity estimates using Daphnia magna. **In**: Aquatic Toxicology and Hazard Assessment. Cardwell RD, Purdy R and Bahner RC (eds), STP 854. American Society for Testing and Materials, Philadelphia, PA. 87-103.

Adams WJ and Renaudette WJ (1983) Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge Paratanytarsus parthenogenetica. Monsanto Industrial Company report ES-81-SS-35.

Adams WJ, Biddinger GR, Robillard KA and Gorsuch JW (1995) A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environ. Toxicol. and Chem. **14** (9), 1569-1574.

Adema et al. (1981) Onderzoek naar een geschikte combinatie toetsmethoden ter bepaling van de aquatische toxiciteit van milieugevaarlijke stoffen. RIVM Report No CL81/100, RIV 627905 001.

Agarwal DK, Eustis S, Lamb IV JC, Jameson CW and Kluwe WM (1986a) Influence of dietary zinc on di(2-ethylhexyl) phthalate-induced testicular atrophy and zinc depletion in adult rats. Toxicol. Appl. Pharmacol. **84**, 12-24.

Agarwal DK, Eustis S, Lamb IV JC, Reel JR and Kluwe WM (1986b). Effects of di(2-ethylhexyl) phthalate on the gonadal pathophysiology, sperm morphology, and reproductive performance of male rats. Environ. Health Perspect. **65**, 343-350.

Agarwal DK, Lawrence J and Autian J (1985b) Antifertility and mutagenic effects in mice from parenteral administration of di-2-ethylhexyl phthalate (DEHP). J. Toxicol. Environ. Health **16**, 71-84.

Agarwal DK, Lawrence WH and Turner JE (1989) Effects of parenteral di(2-ethylhexyl) phthalate (DEHP) on gonadal biochemistry, pathology, and reproductive performance of mice. J. Toxicol. Environ. Health **26**, 39-59.

Agarwal DK, Lawrence WH, Nunez LJ and Autian J (1985a) Mutagenicity evaluation of phthalic acid esters and metabolites in *Salmonella typhimurium* cultures. J. Toxicol. Environ. Health **16**, 61-69.

Akingbemi BT et al. (2001) Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate, Biology of reproduction **65**, 1252-1259.

Al-Badry MS and Knowles CO (1980) Phthalate-organophosphate interactions: Toxicity, penetration and metabolism studies with houseflies. Arch. Environm. Contam. Toxicol. **9**, 147-161.

Alberti J, Brull K, Furtmann G and Braun (2000) Occurrence of phthalates in German Surface and wastewater, Poster presentation at the 10th annual SETAC Europe meeting Brighton, UK. 21-25 May. 5.

Albro PW (1975) The metabolism of 2-ethylhexanol in rats. Xenobiotica **5**, 625-636.

Albro PW (1986) Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. Environ. Health Perspect. **65**, 293-298.

Albro PW, Chae K, Philpot R, Corbett JT, Schroeder J and Jordan S (1984) In vitro metabolism of mono-2-ethylhexyl phthalate by microsomal enzymes. Similarity to omego-and (omega-1) oxidation of fatty acids. Drug Metab. Dispos. **12** (6), 742-748.

Albro PW and Thomas RO (1973) Enzymatic hydrolysis of di-(2-ethylhexyl) phthalate by lipases. Biochem. Biophys. Acta. **360**, 380-390.

Albro PW, Corbett JT, Schroeder JL, Jordan S and Matthews HB (1982a) Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. Environ. Health Perspect. **45**, 19-25.

Albro PW, Hass JR, Peck CC, Odam DG, Corbett JT, Bailey FJ, Blatt HE and Barrett BB (1981) Identification of the metabolites of di-(2-ethylhexyl) phthalate in urine from the african green monkey. Drug Metab. Disp. **9**, 223-225.

Albro PW, Jordan ST, Schroeder JL and Corbett JT (1982b) Chromatographic separation and quantitative determination of the metabolites of di-(2-ethylhexyl) phthalate from urine of laboratory animals. J. Chromatograph. **244**, 65-79.

Albro PW, Thomas R and Fishbein L (1973) Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr. **76**, 321-330.

Albro PW, Tondeur I, Marbury D, Jordan S, Schroeder J and Corbett JT (1983) Polar metabolites of di-(2-ethylhexyl) phthalate in the rat. Biochim. Biophys. Acta. **760**, 283-292.

Alcontrol Biochem Laboratoria (1999) The analysis of phthalates in soil and sediment.

Allwood MC (1986) The release of phthallate ester plasticizer from intravenous administration sets into fat emulsion. Int. J. Pharm. **29**, 233-236.

Allwood MC and Martin H (1996) The extraction of diethylhexylphthalate (DEHP) from polyvinyl chloride components of intravenous infusion containers and administration sets by paclitaxel injection Int. J. Pharm. **127**, 65-71.

Al-Omran LA and Preston MR (1987) The Interactions of Phthalate Esters with Suspended Particulate Material in Fresh and Marine Waters. Env. Poll. **46**, 177-186.

Amacher DE and Turner GN (1985) Tests for gene mutational activity in the L5178Y/TK assay system. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. Progr. Mutat. Res**. 5**, 487-496.

American Society for Testing and Materials (1993) ASTM Standards on Aquatic Toxicology and Hazard Evaluation. ASTM, Philadelphia, USA, ISBN 0-8031-1778-7.

Anderson WAC, Castle L, Scotter MJ, Massey RC and Springall C (2001) Food Add. Cont. 18, 1068-1074 2001. A biomarker approach to measuring human dietary exposure to certain phthalate diesters.

Andrén Claes and Nilsson Göran. Personal communication. Dept. of Zoology, University of Gothenburg, Sweden.

Anonymous (1987) Entwarnung für Fisch. Test 22, Nr. 12, 74-76, cited in ULIDAT Nr. LF875987.

Anzion C (1987) Het voorkomen van ftalaten in het milieu, Free University, Amsterdam, (1978). **In**: The Science of the Total Environ. WamsTJ. **66**, 1-16.

Aranda JM, O´Conner GA and Eiceman GA (1989) Effects of sewage sludge on Di-(2-ethylhexyl)phthalate uptake by plants. J. Environ. Qual. **18**,45-50.

Arbin A, Östelius J, Callmer K, Sroka J, Hänninen K and Axelsson S (1983). Migration of chemicals from soft PVC bags into intravenous solutions. Acta. Pharm. Suecia Suppl. **3**, 20-33.

Arcadi FA, Costa C, Imperatore C, Marchese A, Rapidisarda A, Salemi M, Trimarchi GR and Costa G (1998). Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in Long-Evans rat. Food Chem. Toxicol. **36**, 963-970.

Aristech Chemical Corporation and Eastman Chemical Company (1998). Expert Panel Report on DEHP.

Arni P (1985). Induction of various genetic effects in the yeast *Saccharomyces cerevisiae* strain D7. **In**: Elsevier Science Publ. Ashby J and de Serres FJ (eds). Progr. Mutat. Res. **5**, 217-224.

Ashby J et al. (1994). Mechanistically-based Human Hazard Assessment of Peroxisome Proliferator-induced Hepatocarcinogenesis, Human and Experimental Toxicology. Zeneca Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield Cheshire, SK10 4TJ, UK. **13** (2), 1-117

Ashby J, de Serres FJ, Draper M, Ishidate Jr M, Margolin BH, Matter BE and Shelby MD (1985). Evaluation of short-term tests for carcinogens. Report of the International Programme on Chemical Safety's collaborative study on in vitro assays. Elsevier Science Publ. Progr. Mutat. Res. **5**.

Astill B, Barber E, Lington A, Moran E, Mulholland A, Robinson E and Scheider B (1986). Chemical industry voluntary test program for phthalate esters: Health effects studies. Environ. Health Perspect. **65**, 329-336.

Atlas E and Giam CS (1980) Water, Air and Soil Poll. **38**, 19-36.

Atlas E and Giam CS (1987). Science **211**, 163-165, (1981), **In**: The Science of the Total Environment. Wams TJ **66**, 1-16.

ATSDR (1993) Toxicological Profile for di(2-ethylhexyl) phthalate. TP-92/05. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Auergesellschaft GmbH (Hrsg) (1989) Auer-Technikum, 12 Ausgabe; Berlin. Referenced in Rippen, 2005.

Badr MF (1992) Induction of peroxisomal enzyme activities by di(2-ethylhexyl) phthalate in thyroidectomized rats with parathyroid replants. J. Pharmacol. Exp. Therap. **263**, 1105-1110.

Baker RSU and Bonin AM (1985). Tests with the Salmonella plate-incorporation assay. **In**: Ashby J and de Serres FJ (eds) Elsevier Science Publ. Progr. Mutat. Res. **5**, 177-180.

Baker RWR (1978) Diethethyl phthalate as a foctor in blood transfusion and heamodialysis Toxicology **9**, 319-329.

Barber ED, Astill BD, Moran EJ, Schneider BF, Gray TJB, Lake BG and Evans JG (1987). Peroxisome induction studies on seven phthalate esters. Toxicol. Ind. Health **3**, 7-24.

Barber ED, Teetsel NM, Kolberg KF and Guest D (1992). A Comparative Study of the Rates of in Vitro Percutaneous Absorption of Eight Chemicals Using Rat and Human Skin. Fundamental Appl. Toxicol. **19**, 493-497.

Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, Sadowski M, Needham LL and Calafat AM (2003). Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Env. Health Perspect. **111**, 1148-1151

Barrett JC and Lamb PW (1985) Tests with the Syrian hamster embryo cell transformation assay. **In**: Ashby J and de Serres FJ (eds), Elsevier Science Publ. Progr. Mutat. Res. **5**, 623-628,

Barrows ME, Petrocelli SR, Macek KJ and Carroll JJ (1980) Bioconcentration and elimination of selected water pollutants by Bluegill sunfish (Lepomis macrochirus). E G and G Bionomics Aquatic Toxicology Laboratory, Massachusetts, and US-EPA.

Barry YA, Labow RS, Keon WJ, Tocchi M and Rock G (1989) Perioperative exposure to plasticizers in patients undergoing cardiopulmonary bypass. J. Thorac. Cardiovasc. Surg. **97**, 900-905.

Barton HA and Andersen ME (1997) Dose-response assessment strategies for endocrine-active compounds. Regul. Toxicol. Pharmacol. **25**, 292-305.

BASF (1941) Versuche mit Palatinol AH, C, DP, F, HS, K und Mesamoll I und mit diesen Weichmachern hergestellten Igelit-Folien. BASF AG: dept. of toxicology, unpublished results, 03-10-41.

BASF (1953) Bericht über die biologische Virkung von Palatinol AH. BASF AG: dept. of toxicology, unpublished results (III/211),07-16-53.

BASF (1961) Bericht über die toxikologische Prüfung von Palatinol C, IC, AH, DN und Z. BASF AG: dept. of toxicology, unpublished results (VII/4), 01-10-61.

BASF (1982) Tests for the binding of di(2-ethylhexyl) phthalate (DEHP) to rat liver DNA in vivo. BASF AG: dept. of toxicology, unpublished results (82/1), 06-07-82.

BASF (1986a) Report on the acute dermal irritation/corrosivity to the intact dorsal skin of the White rabbit based on OECD; Report on the acute irritation to the eye of the White rabbit based on OECD. BASF AG: dept. of toxicology, unpublished results (85/380), 02-20-86.

BASF (1986b) Bericht über die Prüfung von Palatinol AH auf praenatale Toxizität (inkl. Aufzucht) an Ratten anch inhalativer Aufnahme des Flüssigkeitsaerosols. BASF AG: dept. of toxicology, unpublished results (82/198), 11-17-86.

BASF (1990) Inhalationstoxizität von di(2-ethylhexyl) phthalat an Wistar-Ratten (28-Tage-Versuch mit 8 wöchiger Nachbeobachtung) und Fertilitätsüberprüfung der behandelten männlichen Wistar-Ratten. BASF AG: dept. of toxicology, unpublished results (85/50), 05-31-90.

BASF (1991) Study of prenatal toxicity of Isodecanol, 2-Ethylhexanol and 711 Alcohol (T.C.) in rats after oral administration (gavage). Project No. 92R0753/88069. EPA Document No. 88-90000082, Microfiche No. OTS0524015 (1990) EPA Document No. 8EHQ-0491-0910S, Microfiche No. OTS0524015-2 (1991).G24

BASF (1995) BASF AG: dept. of toxicology, unpublished results (91/124), 09-28-95.

BASF AG (1960) Bericht über die Prüfung von Aethylhexylphthalat, Dinonylphthalat und Paraffinöl im Fütterungsversuch mit Sprague-Dawley-Ratten. Dept. of toxicology, unpublished results (VII/4).

BASF AG (1983) Sapromattest: Ergebnisuebersicht und Testbewertung vom26.05.1983, Laboratory of Ecology, Unpublished report, DUU/OM -– Z 570, 11 ppg. (in German).

BASF AG (1984) Labor Oekologie, unveroeffentlichte Untersuchung (VCI Projekt schwer wasserloesliche Stoffe), Bericht vom 23.02.1984.

BASF AG (1987) Analytical laboratory, unpublished results, report BRU 87.212 (08.10.1987)

BASF AG (1989) Technical review Palatinol AH, M 2189 d (06/1989)

BASF AG (1990) Bestimmung der Algentoxizität. Labor für Oekologie. Unpublished report Oekonummer 01/89/1026 (in German).

BASF AG (1991) Sauerstoffverbrauchtest. Labor für Oekologie, Oekonummer 01/89/1026, 3 ppg. (Unpublished report in German).

BASF AG (1994a) Sicherheitsdatenblatt Palatinol AH (17.03.1994)

BASF AG (1994b) Safety data sheet Palatinol AH (12.08.1994)

BASF AG (1996) Oekologie und Umweltanalytik, Notiz vom 7.2.96.

BASF AG (1999) Letter to KemI. 25 August 1999.

Basseres A (2000) RESUME: DEHP phtalate analysis in stream. Elf Atochem, project number H50206.

Batelle Institut (1982) Review “Merkblaetter ueber Referenzchemikalien” (11/1982).

Becker W (1989) Befolkningens kostvanor och näringsintag i Sverige 1989. Livsmedelsverket, Sweden

Bell FP (1982) Effects of phthalate esters on lipid metabolism in various tissue cells and organelles in mammals. Environ. Health Perspect. **45**, 41-50.

Bell FP, Patt CC, Brundage B, Gillies PJ and Phillips WA (1978) Studies on lipid biosynthesis and cholesterol content of liver and serum lipoproteins in rats fed various phthalate esters. Lipids **13**, 66-74.

Bentley P, Calder I, Elcombe C, Grasso P, Stringer D and Wiegand H-J (1993) Hepatic peroxisome proliferation in rodents and its significance for humans. Fd. Chem. Toxicol. **31**, 857-907.

Berndtsson B-S (1991) Emission in Sweden of phthalic acid esters (PAE´s) from production and from the processing industry using PAE´s as plasticizers, Neste Chemicals PM 6/91.

Berger MR (1995) Combination effect of three non-genotoxic carcinogens in male SD rats. Proceedings of the American association for cancer research annual meeting. **36** (0), 133.

Bergkvist P et al. (1989). Swedish Environmental Protection Agency. Report No. 3624.ISBN 91-620-3624-6.

Berglind Sven-Åke. Personal communication. Dept. of Genetics, University of Uppsala, Sweden.

Bergstedt O (1996) Miljöföroreningar in dricksvattenförsörjningen -– inledande studie exemplifierad med nonyl/oktylfenol, ftalater och PAH. Göteborgs Vatten- och Avloppsverk, produktionsavdelningen. 1996-04-08.

Berman E and Laskey JW (1993) Altered steroidogenesis in whole-ovary and adrenal culture in cycling rats. Reproductive Toxicology **7**, 349-358.

Bessems E (1988) The Biodegradation of Plasticized PVC and Its Prevetion. J. Vinyl Technology **10** (1), 3-6.

BGAA (1996) (Berufsgenossenschaftlicher Arbeitskreis Altstoffe Bundesrepublik Deutschland). Di-(2-ethylhexyl)-phthalate. Occupational Exposure, Exposure descition no.22.

BIBRA (1990) An investigation of the effect of di-(2-ethylhexyl) phthalate (DEHP) on rat hepatic peroxisomes. BIBRA-Report No. 826/2/90, 01-90.

Bionomics INC. (1972) Acute toxicity of compounds to bluegill (Lepomis macrochirus). Submitted to USEPA. Doc.I.D. 878220808.

Birge W, Black and Westerman A (1978) Effects of polychlorinated biphenyl compounds and proposed PCB-replacement products on embryo-larval stages of fish and amphibians. Water Resources Research Institute, Kentucky University, Lexington, Kentucky. Research report No. **118**, 39.

Birge WJ, Black JA and Bruser DM (1979) Toxicity of organic chemicals to embryo-larval stages of fish. University of Kentucky Water Resources Research Institute, Lexington Kentucky. Contrac number 68-01-4321. Final report.

Bis(2-ethylhexyl) Phthalate, Priority Substances List Assessment Report, Canadian Environmental Protection Act, 1994.

Blass CR, Jones C and Coutney JM (1992). Biomaterials for blood tubing: the application of plasticised poly(vinyl chloride). Int. J. Artif. Organs. **15**, 220-203.

Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ and Brock JW (2000). Levels of Seven Urinary Phthalate Metabolites in a Human Reference Population. Environ. Health Perspect. **108** (10), 972-982.

Bodar C (1996) Letter from Charles Bodar RIVM (25 March, 1996) referring some Dutch monitoring data on water, sediment and sewage sludge.

Bodar C. (1997) Letter from Charles Bodar RIVM.

Bommer J, Ritz E and Andrassy K (1985) Side effects due to materials used in hemodialysis equipment. Adv. Nephrol. **14**, 409-438.

Bommer J, Waldherr R, Gastner M, Lemmes R and Ritz (1981). Lactogenic multiorgan silicone inclusions in dialysis patients. Klin. Wochenschr. **59**, 1149-1157.

Boutrup S, Erichsen PC, Wiggers L and Jensen A (1998): Miljöfremmende stoffer in Århus Amt -– fase 2 og 3, 1997-1998. Århus Amt Natur og Miljökontoret, Lyseng Allé, DK-8270 Höjbjerg, Denmark.

Bower RK, Haberman S and Minton PD (1970) Teratogenic effects in the chick embryo caused by esters of phthalic acid. J. Pharmacol. Exp. Ther. **171**, 314-324.

BP Chemicals (1996) Environmental assessment of Di-2-Ethyl Hexyl Phthalate (DEHP) production at BP Chemicals Ltd, Hull Site.

BP Chemicals (1999). Occupational Hygiene Monitoring Report. BP Chemicals Limited Hull Site.

Braaten et al. (1996). NIVA Report SNO 3552-96, ”Occurrence of phthalates and organotins in sediments and water in Norway”

Bradley MO (1985) Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes. **In**: Ashby J and de Serres FJ (eds) Elsevier Science Publ. Progr. Mutat. Res. **5**, 353-357.

Branzén H (2000) Monitoring data in ground water. Letter from “Länsstyrelsen Blekinge län”.

Bringmann G and Kühn R (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. **14**, 231-241.

Bringmann G and Kühn R (1981) Vergleich der wirkung von schadstoffen auf flagellate sowie ciliate bzw. auf holozoiche bakterienfressende sowie saprozoische Protozoen. Gas- und Wasserfach -– Wasser/Abwasser. **122**, 308-313.

Brock JW, Caudill SP, Silva MJ, Needham LL and Hilborn ED (2002) Phthalate monoesters levels in the urine of young children. Bull. Environ. Contam. Toxicol. **68**, 309-314.

Bronsch CJT (1987) Untersuchungen zur Exposition und zum renalen Ausscheidungsverhalten des Kunststoffweichmachers di-(2-ethylhexyl)phthalat (DEHP) beim Menschen. Dissertation No. 8459, Eidgenoessische Technische Hochschule, Zuerich, Switzerland.

Brooke et al. (1990) Chemosphere **21**, 119-133.

Brown D and Thompson RS (1982a) Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexylphthalate (DEHP) and di-isodecyl phthalate (DIDP) on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere **11** (4), 417-426.

Brown D and Thompson RS (1982b). Phthalates and the aquatic environment: Part II. The bioconcentration and depuration of di-2-ethylhexylphthalate (DEHP) and di-isodecyl phthalate (DIDP) in mussels (Mytilus edulis). Chemosphere **11** (4), 427-435.

Brown D, Croudace CP, Williams NJ, Shearing JM and Johnson PA (1998) The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere **36** (6), 1367-1379.

Bruns-Weller E and Pfordt J (2000) Bestimmung von Phthalsäureestern in Lebensmitteln und Frauenmilch. ERNO **1** (1), 25-28.

Brunetti G and Moscato G (1984) Bronchial asthma due to occupational exposure to a dioctylphtalate. Description of a case. Med. Lav. **75**, 120-124.

BUA (1986). Di-(2-ethylhexyl) phthalate. BUA Report 4. January 1986. Gesellschaft Deutscher Chemiker.

Buccafusco RJ, Ells SJ and LeBlanc GA (1981) Acute toxicity of priority pollutants to Bluegill (Lepomis macrochirus). Bull. Environm. Contam. Toxicol. **26**, 446-452.

Buchen G und Vogel U (1995) Ökotoxizität von Di-(2-ethylhexyl)-phthalate -– Akuter Daphnientest (24h/48h) mit Daphnia magna nach DIN 38412 Teil 11. Institut für Wasser-, Boden u. Lufthygiene Umweltbundesamt, Berlin.

Building Research Establishment (1994 and amended version of 1997). Use Category Document. Plastic Additives.

Burns BG, Musial CJ and Uthe JF (1981) Novel cleanup method for quantitative gas chromatographic determination of trace amounts of Di-2-ethylhexyl phthalate in fish lipid. J. Assoc. Off. Anal. Chem. **64**, 282-286.

Burton GA (Jr) (1992). Sediment toxicity assessment. Lewis Publishers Inc., Chelsea, USA, 457.

Büsser M-T and Lutz WK (1987) Stimulation of DNA synthesis in rat and mouse liver by various tumor promoters. Carcinogenesis **8**, 1433-1437.

Butterworth BE, Bermudez E, Smith-Oliver T, Earle L, Cattley R, Martin J, Popp JA, Strom S, Jirtle R and Michalopoulos G (1984). Lack of genotoxic activity of di(2-ethylhexyl) phthalate (DEHP) in rat and human hepatocytes. Carcinogenesis **5**, 1329-1335.

Butterworth BE, Smith-Oliver T, Earle L, Loury DJ, White RD, Doolittle DJ, Working PK, Cattley RC, Jirtle R, Michalopoulos G and Strom D (1989). Use of primary cultures of human hepatocytes in toxicology studies. Cancer Res. **49**, 1075-1084.

Cadogan DF, Papez M, Poppe AC, Pugh DM and Scheubel (1994). An Assessment of the Release, Occurrence and Possible Effects of Plasticisers in the Environment, Progress in Rubber and Plastics Technology. Buist Dr. JM (ed), Rapra Technology **10** (1).

Calafat AM, Slakman AR, Silva MJ, Herbert AR and Needham LL (2004) J. Chromatography B, **805**, 49-56, Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites

Caldwell DJ (1999). Review of mononuclear cell leukemia (MNCL) in F-344 rat bioassays and its significance to human cancer risk: a case study using alkyl phthalates. Regul. Toxicol. Pharmacol. **30** (1), 45-53.

Caldwell J, Notarianni LJ, Smith RL, Fafunso MA, French MR, Dawson P and Bassir O (1979a) Non-human primates as metabolic models for the human situation. Comparative studies on meperidine metabolism. Toxicol. Appl. Pharmacol. **48**, 273-278.

Caldwell J, O`Gorman J, Smith RL, Bassir O Fafunso MA and French MR (1979b). Monkeys as metabolic models for man – the fate of amphetamine in the vervet and patas monkey. Br. J. Pharmacol. **66**, 156-157.

Canadian Water Quality Guidelines: Updates (October 1993). Appendix XIII, Three phthalate esters. Page XIII-18.

Canton JH, Adema DMM et al. (1984) Ondersoek naar een geschikte combinatie toetsmethoden ter bepaling van de aaquatische toxiciteit van milieugevaarlijke stoffen. Rapport nr.:81/100A RIVM 668114-002.

Carere A, Conti L and Crebelli R (1985) Assays in *Aspergillus nidulans* for the induction of forward-mutation in haploid strain 35 and for mitotic nondisjunction, haploidization and crossing-over in diploid strain P1. **In**: Ashby J and de Serres FJ (eds) Elsevier Science Publ. Progr. Mutat. Res. **5**, 307-312.

Carls N and Schiestl RH (1994) Evaluation of the yeast DEL assay with 10 compounds selected by the International Program on Chemical Safety for the evaluation of short-term tests for carcinogens. Mutat. Res. **320**, 293-303.

Cartwright CD, Thompson IP and Burns RG (2000) Degradation and impact of phthalate plasticizers on soil microbial communities. Environ. Toxicol. Chem. **19**, 1253-1261.

Castle L, Gilbert J and Eklund T (1990) Migration of plasticiser from poly(vinyl chloride) milk tubing. Food Addit. Contam. **7** (5), 591-596.

Castle L, Mayo A and Gilbert J (1989) Migration of plasticizers from printing inks into foods, Food Addit. Contam. **6**, 437-443.

Castle L (2003) Central Science Laboratory, York UK. Personal communication. Letter to KEMI by e-mail. 7th October 2003

Cattley RC and Popp JA (1989) Differences in the promoting activities of the peroxisome proliferator Wy-14,643 and phenobarbital in rat liver. Cancer Res. **49**, 3246-3251.

Cattley RC, Conway JG and Popp JA (1987) Association of persistent peroxisome proliferation and oxidative injury with hepatocarcinogenicity in female F-344 rats fed di-(2-ethylhexyl) phthalate for 2 years. Cancer Lett. **38**, 15-22.

Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tuggwood J and Wahli W (1998). Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? Reg. Toxicol. Pharmacol. **27**, 47-60.

Caunter JE, Williams TD and Shillabeer N (2004), Di-2-Ethylhexylphtalate: Multi-generation study with the fathead minnow (*Pimephales promelas*). Sudy No AJ0172. Performed by Brixham environmental laboratory Astra Zeneca UK Ltd, sponsored by European Council for Plasticisers and Intermediates.

Cears RF and Poppe AC (1993) Weichmacher. Kunststoffe **83**, 10.

CEC (1993). Synoptic document No. 6, draft provisional of monomers and additives used in the manufacture of plastics and coatings intended to come into contact with foodstuffs. Commmision of the European Communities document CS/PM/2064, 2 April 1993.

CEFIC (1982) Report to CEFIC on a 28 day dose and time response study of di(2-ethylhexyl) phthalate in rats. Univ. of Surrey, Robens Institute of Industrial and Environmental Health and Safety, unpublished results, study no. 5/81/TX, 05-21-82.

CEFIC (1997) An estimation of the evaporation of DEHP from WWTP Comment sludge spread on land. draft report. June 1997.

Charles River Laboratories, Portage, Michigan.

Charney RL, Ryan JA and O´Conner GA (1991). risk assessment for organic micropollutants. U.S. point of view. Technical report EPA/600/D-91/062.

Chernoff N and Kavlock RJ (1982). An *in vivo* teratology screen utilising pregnant mice. J. Toxicol. Environ. Health **10**, 541-550.

Chikae M, Hatano Y, Ikeda R, Morita Y, Hasan Q and Tamiya E (2004a). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Pharmacol. **16** (3), 141-145.

Chikae M, Ikeda R, Hatano Y, Hasan Q, Morita Y and Tamiya E (2004b). Effects of bis(2-ethylhexyl) phthalate,γ-hexachlorocyclohexane, and 17^{β}-estradiol on the fry stage of medaka (*Oryzias latipes*). Environ. Toxicol. Pharmacol. **18** (1), 9-12.

Chu I, Dick D, Bronaugh R and Tryphonas L (1996). Skin reservior formation and bioavailabilty of dermally administered chemicals in hairless guinea pigs. Fd. Chem. Toxic. **34**, 267-276.

Chu I, Villeneuve DC, Secours V, Franklin C, Rock G and Viau A (1978) Metabolism and tissue dsistribution of mono-2-ethylhexyl phthalate in the rat. Drug Met. Disp. **6** (2), 146-149.

Chu I, Secours VE, Marino IA, Villeneuve DC and Valli VE (1981). Sub-acute and sub-chronic toxicity of mono-2-ethylhexyl phthalate in the rat. Arch. Environ. Contam. Toxicol. **10**, 271-280.

Clausen PA, Wolkoff P and Svensmark B (1999) Preliminary Study of Semivolatile Organic Compounds in Some Danish Indoor Environments. Proceedings of the 8th International Coference on Indoor Air Quality and Climate held in Edinburgh, Scotland, 8-13 August 1999.

Clayton GD and Clayton FE (1981) Patty's industrial hygiene and toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed New York, John Wiley Sons. 2344.

CLEA (2000). Personal communication concerning the sexually maturity of male marmosets. CLEA Japan, Inc

Clegg ED, Cook JC, Chapin RE, Foster PM and Daston GP (1997) Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproductive Toxicology; **11** (1), 107-121.

CMA (1982a) DEHP metabolism studies. Dose dependence of and effect of prior exposure on the metabolism of DEHP administered in the diet to rats. TSCATS: 0286, Doc. I. D.: FYI-AX-0384-0286SU, 05-01-82, Chemical Manufacturers Association.

CMA (1982b) DEHP metabolism studies. Species differences in the metabolism of a single oral dose of DEHP. TSCATS (1982d). TSCATS: OTS 0508486, Doc. I. D.: 40-8426085, 01-01-82, Chemical Manufacturers Association.

CMA (1982c) Toxicological effects of di(2-ethylhexyl) phthalate. TSCATS: OTS 00000224-0, Doc. I. D.: FYI-AX-1282-0224, 10-30-82, Chemical Manufacturers Assoc.

CMA (1982d) Mutagenicity evaluation of di(2-ethylhexyl) phthalate in the Ames Salmonella/microsome plate test. TSCATS: OTS 0520393, Doc. I. D.: 86-890000955, 07-01-82, Chemical Manufacturers Assoc.

CMA (1983) DEHP metabolism studies. Species differences in the metabolism of a single oral dose of DEHP. TSCATS: 0286A, Doc. I. D.: FYI-OTS-1084-0286SU, 04-30-83, Chemical Manufacturers Association.

CMA (1984a) DEHP metabolism studies. Species differences in the metabolism of a single oral dose of DEHP. TSCATS: OTS 0508494-1, Doc. I. D.: 40-8426135, 09-04-84, Chemical Manufacturers Association.

CMA (1984b) A 21 day dose-relationship study of di(2-ethylhexyl) phthalate in rats. TSCATS: OTS 0508501, Doc. I. D.: 40-8526196, 07-01-84, Chemical Manufacturers Assocication; TSCATS: OTS 0509537, Doc. I. D.: 40-8526206, 07-01-84, Chemical Manufacturers Association; TSCATS: OTS 0536220, New Doc. I. D.: 88-920002026, 07-01-84,Chemical Manufacturers Association.

CMA (1984c) Chemical Manufacturers Assoc.: Assessment of possible carcinogenic risk resulting from exposure to di(2-ethylhexyl) phthalate (DEHP) in children's products, 07-09-84

CMA (1985) Evaluation of di(2-ethylhexyl) phthalate (DEHP) in the CHO/HGPRT forward mutation assay. TSCATS: OTS 0508501, Doc. I. D.: 40-8526196, 02-01-85, Chemical Manufacturers Assoc.

CMA (1989) How to comply with proposition 65: DEHP exposure. Chemicals Manufacturers Association, Washington, DC.

CMA (1997) Research program: Toxicity of phthalate esters in sediments, program summary. Chemicals Manufacturers Association, Washington, DC. 3.

Cocchieri RA (1986) Occurrence of Phthalate Esters in Italian Packaged Foods. J. Food Protect. **49**, 265-266.

Cohen H, Charrier C and Sarfaty J (1991) Extraction and identification of a plasticizer, di-(2-ethylhexyl)phthalate, from a plastic bag containing contaminated corn, Arch. Environ. Contam. Toxicol. **20**, 437-440.

Cohle P and Stratton J (1992) Early life-stage toxicity of DEHP (CAS No. 117-81-7) to Rainbow trout (Onchorhynchus mykiss Walbaum 1792) in a flow-through system. ABC inc.. Final report #36568. Sponsored by BASF AG. BASF project no. 52FO7822/875236.

Colón I, Caro D, Bourdony CJ and Rosario O (2000) Identification of phthalates esters in the serum of young Puerto Rican girls with premature breast development. Environ. H. Perspect. **108**, 895-900

Condea (1995) Partition coefficient n-octanol/water and water solubility of phthalic acid esters, Bunsbuettel. Condea Report Sh/94-24, (18.09.1995).

Conway JG, Tomaszewski KE, Olson MJ, Cattley RC, Marsman DS and Popp JA (1989). Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl) phthalate and Wy-14643. Carcinogenesis **10**, 513-519.

Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM and Foster PMD (1999) Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Crit. Rev. Toxicol. **29**, 169-261

Courtens JL and Plöen L (1999) Improvement of spermatogenesis in adult chryptorchid rat testis by intratesticular infusion of lactate. Biol. Reprod. Jul; **61** (1),154-161.

Cox GV and Moran EJ (1984) Summary of environmental fate and effects database on 14 phthalate esters. Summary report -– environmental studies -– phase I. Phthalate Esters Program Panel, Chemical Manufacturers Association, Washington, D.C..

CPSC (1985) U.S. Consumer Product Safety Commisson. Report to the Consumer Product Safety Commisson by the Chronic Hazard Advisory Panel on Di(2-ethylhexyl)phthalate (DEHP). U.S. Consumer Product Safety Commisson, Washington D.C.

Creasy DM, Foster JR and Foster PMD (1983). The morphological development of di-*n*-pentyl phthalate induced testicular atrophy in the rat. J. Pathol. **139**, 309-321.

Crespi CL, Ryan CG, Seixas GM, Turner TR and Penman BW (1985) Tests for mutagenic activity using mutation assays at two loci in the human lymphoblast cell lines TK6 and AHH-1. **In**: Progr. Mutat. Res. Ashby J and de Serres FJ (eds) Elsevier Science Publ. **5**, 497-516.

CRC (1995) Handbook of Chemistry and Physics, 76:th ed Boca Raton, Fl: CRC Press Inc. 3-38.

Crocker JF, Safe SH and Acott P (1988) Effects of chronic phthalate exposure on the kidney. J. Toxicol. Environ. H. **23** (4), 433-444.

CSCL (1992) Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, edited by Chemicals Inspection and Testing Institute Japan, published by Japan Chemical Industry Ecology-Toxicology and Information Center, October 1992.

CSTEE (1998a) EU Scientific Committe on Toxicity, Ecotoxicity and the Environment. Phthalate migration from soft PVC toys and child-care articles. Opinion expressed at the CSTEE third plenary meeting Brussels, 24 April 1998.

CSTEE (1998b) EU Scientific Committe on Toxicity, Ecotoxicity and the Environment. Opinion on Phthalate migration from soft PVC toys and child-care articles - Data made available since the 16th of June 1998, opinion expressed at the 6th CSTEE plenary meeting, Brussels, 26/27 November 1998.

Curto KA and Thomas JA (1982) Comparative effects of diethylhexyl phthalate or monoethylhexylphthalate on male mouse and rat reproductive organs. Toxicol. Appl. Toxicol. **62**, 121-125.

Dalgaard et al. (2001) Toxicology Letters, 122, 69-79, The acute effects of mono(2-ethylhexyl)phthalate (MEHP) on testes of prepubertal Wistar rats

Dalgaard M et al. (2000) Toxicity Study of Di(2-Ethylhexyl)phthalate (DEHP) in Combination with Aceton in Rats, Pharmacol. Toxicol. **86**, 92-100.

Daniel JW and Bratt H (1974) The absorption, metabolism and tissue distribution of di-(2-ethylhexyl) phthalate in rats. Toxicol. **2**, 51-65.

Davis BJ, Maronpot RR and Heindel JJ (1994a) Di(2-ethylhexyl) phthalate suppresses oestradiol and ovulation in cycling rats. Toxicol. Appl. Pharmacol. **128**, 216-223.

Davis BJ, Weaver R, Gaines LJ and Heindel JJ (1994b) Mono-(2-ethylhexyl) phthalate suppresses oestradiol production independent of FSH-cAMP stimulation in rat granulosa cells. Toxicol. Appl. Pharmacol. **128**, 224-228.

Davis JA (1981) Comparison of static-replacement and flow-through bioassays using duckweed, Lemna gibba G-3. US EPA, Report. No. 560/6-81-003, 89.

De Bruijn et al. (1989) Environ. Toxicol. Chem. **8**, 499-512.

De Coste JB (1968) Soil Burial resistance of vinyl chloride plastics. I and EC Product Research and Development. Vol. 7 No. 4 December 1968.

De Coste JB (1972) Effects of Soil Burial Exposure on the Properties of Plastics for Wire and Cable. American Telephone and Telegraph Company. The Bell System Technical J. **51** (1).

de Duve C (1983) Microbodies in the living cell. Sci. Am. **248**, 52-62.

Dees, Gazouli and Papadopoulos (2001) Reproductive Toxicology, Effects of mono-ethylhexylphthalate on MA-10 Leydig tumor cells, **15**, 171-187.

DeFoe DL, Holcombe GW and Hammermeister DE (1990) Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environ. Toxicol. Chem. **9**, 623-636.

DEHP in medizinischen Gegenständen aus Kunststoff (PVC), english translation. Bundesgesunheitsblatt No. 2/89 (Federal German Health Gazette).

Deiseri P, Lepri L and Checchini L (1991) Identification and determination of organic compounds in antartic sediments. Annali di Chimica, **81**, 595-603.

Deisinger PJ, Perry LG and Guest D (1998) In Vivo Percutanous Absorption of [^{14}C]DEHP from [^{14}C]DEHP-plasticized Polyvinyl Chloride Film in Male Fischer 344 rats. Food Chem. Toxicol. **36**, 521-527.

Diefenbach (1998a) Bestimmung der auswirkungen vo VestinolAH auf das wachstum terrestricher pflanzen. Abschlussbericht PF-56. Hüls Infractor Gmbh.

Diefenbach (1998b) Bestimmung der auswirkungen vo VestinolAH auf regenwürmer (Eisenia foetida foetida). Abschlussbericht RW-071. Hüls Infractor Gmbh.

Dine T, Luyckx M, Cazin M, Brunet C, Cazin JC and Goudaliez F (1991) Rapid determination by high performance liquid chromatography of di-2-ethylhexyl phthalate in plasma stored in plastic bags.Biomed Chromatogr. **5** (2), 94-97.

Dirven HAAM, van den Broek PHH, Arends AMM, Nordkamp HH, de Lepper AJGM, Henderson PTh and Jongeneelen FJ (1993a) Metabolites of the plasticizer di-(2-ethylhexyl) phthalate in urine samples of workers in polyvinylchloride processing industries. Int. Arch. Occup. Environ. H. **64**, 549-554.

Dirven HAAM, van den Broek PHH and Jongeneelen FJ (1993b) Determination of four metabolites of the plasticizer di-(2-ethylhexyl) phthalate in human urine samples. Int. Arch. Occup. Environ. H. **64**, 555-560.

Dirven HAAM, van den Broek PHH, Peeters MCE, Peters JGP, Mennes WC, Blaauboer BJ, Noordhoek J and Jongeneelen FJ (1993c) Effects of the peroxisome proliferator mono(2-ethylhexyl) phthalate in primary hepatocyte cultures derived from rat, guinea pig, rabbit and monkey. Biochem. Pharmacol. **45**, 2425-2434.

Dirven HAAM, Theuws JLG, Jongeneelen FJ and Bos RP (1991) Non-mutagenicity of 4 metabolites of di(2-ethylhexyl) phthalate (DEHP) and 3 structurally related derivatives of di(2-ethylhexyl) adipate (DEHA) in the Salmonella mutagenicity assay. Mut. Res. **260**, 121-130.

DiVincenzo GD, Hamilton ML, Mueller KR, Donish WH and Barber ED (1985). Bacterial mutagenicity testing of urine from rats dosed with 2-ethylhexanol derived plasticizers. Toxicol. **34**, 247-259.

Doelman CJA, Borm PJA and Bast A (1990) Plasticisers, another burden for asthmatics? Agents and Actions **31,** 81-84.

Dörfler U, Haala R, Matthies M and Scheunert I (1996) Mineralization Kinetics of Chemicals in Soils in Relation to Environmental Conditions. Ecotox. Env. Safety **34**, 216-222.

Dostal LA, Chapin RE, Stefanski SA, Harris MW and Schwetz BA (1988) Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl) phthalate and the recovery of fertility as adults. Toxicol. Appl. Pharmacol. **95**, 104-121.

Dostal LA, Jenkins WL and Schwetz BA (1987b). Hepatic peroxisome proliferation and hypolipidemic effects of di(2-ethylhexyl) phthalate in neonatal and adult rats. Toxicol. Appl. Pharmacol. **87**, 81-90.

Dostal LA, Weaver RP and Schwetz BA (1987a) Transfer of di(2-ethylhexyl) phthalate through rat milk and effects on milk composition and the mammary gland. Toxicol. Appl. Pharmacol. **91**, 315-325.

Douglas GR, Blakey DH, Liu-Lee VW, Bell RDL and Bayley JM (1985) Alkaline sucrose sedimentation, sister-chromatid exchange and micronucleus assays in CHO cells. **In**: Progr. Mutat. Res. Ashby J and de Serres FJ (eds) Elsevier Science Publ. **5**, 359-366,

Douglas GR, Hugenholtz AP and Blakey DH (1986) Genetic toxicology of phthalate esters: mutagenic and other genotoxic effects. Environ. Health Perspect. **65**, 255-262.

Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, Williams G and van Gemert M (1999) A Cancer Risk Assessment of Di(2-ethylhexyl)phthalate: Application of the new US EPA Risk Assessment Guidelines. Regulatory Toxicology and Pharmacology **29**, 327-357.

Dumpert K (1981) Die embryonalentwicklung des krallenfrosches (Xeonopus laevis) als biologisches prescreening -– system für embryotoxische wirkungen von umveltchemikalien. Batelle -– Institut e.V. Frankfurt am Mian, Forschungsbericht 106 03 017, BleV-R-64.493-4, 52.

Dumpert K and Zietz E (1983) Platanna (Xeonopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxicol. Environ. Safety **8**, 55-74.

Dybing E and Sanner T (1997) Evaluation of liver carcinogenicity in rats and mice. In a letter to the Swedish Chemicals Inspectorate, August 31, 1997.

Eagon PK, Chandar N, Epley MJ, Elm MS, Brady EP, and Rao KN (1994) Di(2-ethylhexyl) phthalate-induced changes in liver oestrogen metabolim and hyperplasia. Int. J. Cancer **58**, 736-743.

Eagon PK, Elm MS, Epley MJ, Shinozuka H and Rao KN (1996). Sex steroid metabolism and receptor status in hepatic hyperplasia and cancer in rats. Gastroenterology **110** (4), 1199-1207.

Easterling RE, Johnson E and Napier EA (1974) plasma extraction of plastisizers from "medical grade" polyvinylchloride tubing (38389). **In**: NTIS, PB-260406, 09-76 Proc. Soc. Exp. Biol. Med. **147**, 572.

Eastman Kodak (1984b) Bacterial mutagenicity testing of urine from rats dosed with 2-ethylhexanol derived plasticizers. TSCATS: 206391, Doc. I. D.: 878213941, 01-17-84, Eastman Kodak Co.

Eastman Kodak (1992a) A subchronic (13-week) dietary oral toxicity study of di(2-ethylhexyl) phthalate in Fischer 344 rats (final report). TSCATS: OTS 0530371, Doc. I. D.: 89-910000085, 03-14-91, Eastman Kodak Co.

Eastman Kodak (1992b) A subchronic (4-week) dietary oral toxicity study of di(2-ethylhexyl) phthalate in B6C3F1 mice (final report). TSCATS: OTS 0535432, Doc. I. D.: 86-920000874, 02-19-92, Eastman Kodak Co.

Eastman Kodak (1983) Disposition of [2-hexyl^{14}C]di(2-ethylhexyl) phthalate in the rat. TSCATS: OTS 0206634, Doc. I. D.: 878214716, 06-20-83, Eastman Kodak Co.

Eastman Kodak (1984a) Disposition of [2-hexyl^{14}C]di(2-ethylhexyl) phthalate in the rat. TSCATS: OTS 0508495, Doc. I. D.: 40-8426136, 09-17-84, Eastman Kodak Co.

EC-Commission (1991) Directorate General for Industrial Affairs and International Market, Consolidated report of the scientific committee for food on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs, CS/PM/ 1023,

ECETOC (1985) Technical Report Nr.19, ECETOC Bruessel.

ECETOC (1992) Hepatic peroxisome proliferation. Monograph No. 17. European Chemical Industry Ecology and Toxicology Centre, Brussels.

ECETOC (1996) Monograph No. 26. Aquatic toxicity testing of sparingly soluble, volatile and unstable substances. Brussels, September 1996, ISSN-0773-6047-26, 67.

ECPI (1996a) Plasticiser consumtion and end-use pattern in Western Europe. European Council for Plasticisers and Intermediates. P.Legrand 18.03.1996.

ECPI (1996b) European Council for Plasticisers and Intermediates. Assessment of the release, occurrence and possible effects of plasticisers in the environment. Phthalate esters used in plasticised PVC. Cefic, September 1996.

ECPI (1997) An estimate of the evaporation of DEHP from WWTP sludge spread on land (draft). Letter from European Council for Plasticisers and Intermediates.

ECPI (1998a) Site specific emission data from production in EU. October 1998 (Confidential).

ECPI (1998b) ECPI Position and Response to KemI Preliminary Draft DEHP Risk Assessment Site specific emission data from production in EU. November 1998.

ECPI (1999) ECPI Position and Response to KemI Preliminary Draft DEHP Risk Assessment Site specific emission data from production in EU. November 1998. Final version.

ECPI (2000) Draft EU Risk Assessment DEHP dated May 2000. Comments on draft report. Letter 14 August 2000.

ECPI (2000b) Draft EU Risk Assessment DEHP dated nov. 1999. Comments on draft report. Letter 20 January 2000.

ECPI (2000c) Determination of the influence of Di-2 ethylhexyl phthalate (DEHP) on soil quality: Determination of nitrogen mineralization in soils and the influence of DEHP on these processes; Part II: Determination of dehydrogenase activity; and, Part III: Determination of soil micro flora activity.

ECPI (European Council for Plasticisers and Intermediates) (1994) Medical Applications of Plasticised PVC.

ECPI (European Council for Plasticisers and Intermediates) (1996) Assesment of the release, occurence and possible effects of plasticisers in the environment. Phthalate esters used in plasticised PVC. European Council for Plasticisers and Intermediates.

Eder G (1987). Organische Umweltchemikalien in marinen Sedimenten, Veroeffentlichungen des Instituts fuer Meeresforschung Bremerhafen, **10**, 41-48 (1984). **In**: The Science of the Total Environment. Wams TJ (ed) **66**, 1-16.

Edlund C, Ganning AE and Dallner G (1986). The influence of prolonged di(2-ethylhexyl)phthalate treatment on the dolichol and dolicyl-P content of rat liver. Che.-Biol. Interactions **57**, 255-270.

EG and G Bionomics (1977a) Accumulation and elimination of 14C-residues by fish exposed to 14C-DEHP in LL-1131. Bionomics Doc ID: 878210522. Toxicity test report submitted to General Electric Co., Capacitor Prods. Dept., Hudson Falls, NY.

EG and G Bionomics (1977b) Accumulation and elimination of 14C-residues by fish exposed to 14C-DEHP in LL-1132. Bionomics Doc ID: 878210520. Toxicity test report submitted to General Electric Co., Capacitor Prods. Dept., Hudson Falls, NY.

EG and G Bionomics (1983a) Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions. Bionomics Report #BW-83-3-1373. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

EG and G Bionomics (1983b) Acute toxicity of thirteen phthalate esters to Bluegill (Lepomis macrochirus). Bionomics Report #BW-83-3-1368. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

EG and G Bionomics (1983c) Acute toxicity of thirteen phthalate esters to the fathead minnow (Pimephales promelas) under flow through conditions. Bionomics Report #BW-83-3-1374. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

Egestad B and Sjöberg P (1992) Glucosidation as a new conjugation pathway for metabolites of bis(2-ethylhexyl) phthalate. Drug Metabol. Dispos. **20**, 470-472.

Egestad B, Green G, Sjöberg P, Klasson-Wehler E and Gustafsson J (1996). Chromatographic fractionation and analysis by mass spectrometry of conjugated metabolites of bis(2-ethylhexyl) phthalate in urine. J. Chromatogr. **677**, 99-109.

Ejlertsson J (1997) Fate of Phthalic Acid Esters During Digestion of Municipal Solid Waste Under Landfill Conditions. Thesis. ISBN 91-7871-958-5.

Ejlertsson J and Svensson BH (1997). Degradation of bis(2-ethylhexyl)phthalate constituents. Biodegradation **00**, 1-6.

Ejlertsson J, Meyerson U and Svensson BH (1996) Anaerobic degradation of Phthalic Acid Esters During Digestion of Municipal Solid Waste Under Landfilling Conditions. Biodegradation **7**, 345-352.

Elcombe CR, Bell DR, Elias E, Hasmall SC and Plant NJ (1997) Peroxisome proliferators: species differences in response of primary hepatocyte cultures. Annals New York Acad. Sci. **804**, 628-635.

Elf Atochem (1997). Lemaire, P. Analyse de phthalates dans l'estuarie de la Seine. Ref: 97/SAE8/0544/PL

Ellington JJ and Floyd TL (1996). Octanol/water partition coefficients for eight phthalate esters. US-EPA, Env. Res. Brief. EPA/600/S-96/006.

Elmore E, Korytynski EA and Smith MP (1985). Tests with the Chinese hamster V79 inhibition of metabolic cooperation assay. **In**: Progr. Mutat. Res. Ashby J and de Serres FJ (eds), Elsevier Science Publ. **5**, 597-612.

Elsisi AE, Carter DE and Sipes IG (1989). Dermal Absorption of Phthalate Diesters in Rats. Fund. Appl. Toxicol. **12**, 70-77.

Emissionen im Kraftfahrzeuginnenraum (1998). In ECPI. Position and response to the draft risk assessment bis(2ethylhexylphthalate (DEHP). Part II – Human health. May 1999.

Environ Corporation (1988) Indoor DEHP Air Concentration Predicted after DEHP Volatilizes from Vinyl Products. Prepared for Chemical Manufacturers Association.

Environment Agency Japan (1981) Background paper on the environmental Monitoring of chemical substances in Japan: in Umweltbundesamt (Hrsg.), Proc. Workshop on the control of existing chemicals under the patronage of the OECD. Berlin, 10-12 June. 165-189.

Environment Agency Japan (1995) Chemicals in the environment. March 1995. Report on environmental survey and wildlife monitoring of chemicals. Environmental Health and safety division.

Eriksson P and Darnerud PO (1985) Distribution and retention of some chlorinated hydrocarbons and a phthalate in the mouse brain during the pre-weaning period. Toxicology **37**, 189-203.

Estep TN, Pedersen RA, Miller TJ and Stupar KR (1984) Characterisation of erythocyte quality during the refrigerated storage of whole blood containing di-(2-ethylhexyl)phthalate. Blood **64** (6), 1270-1276.

ETSU (1996) Economic Evaluation of the Draft Incineration Directive. Report produced for the EU DGXI. Contract No. B4 3040/95/001047/MAR/B1. Dec. 1996.

EuPC (European Plastics Converters) EuPC survey on emission of PVC plasticizers during processing/formulation; intermediate report of 8 June 2005

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (1994). Assessment of Non-Occupational Exposure to Chemicals. Technical Report No 58, May 1994.

Exxon (1982a) One-week prechronic oral feeding study in F-344 rats. Pathology report. TSCATS: 206292, Doc. I. D.: 878210916, 07-21-82, Exxon Chemicals Americas.

Exxon (1982b) One-week prechronic oral feeding study TSCATS: 206292, Doc. I. D.: 878210917, 11-19-82, Exxon Chemical Americas.

Exxon (1986a) Chronic toxicity/carcinogenicity study of diisononyl phthalate (DINP) in the rat. Exxon Corporation.

Exxon (1986b) DINP chronic study in rats -statistical methods and results. Exxon Corporation.

Exxon (1994) Jayflex DEHP. Skin sensitisation in the Guinea-pig. Huntingdon Research Centre Ltd., Cambridgeshire (England), unpublished results for Exxon Chemical Int. Inc., Bruxelles (Belgium), Report No. EXX 12d/940605/SS, 11-01-94.

Facts (1994) Health, Safety and the Environment, BP Chemicals.

Fairbanks BC, O´Conner GA and Smith SE (1985) Fate of Di-2-(ethylhexyl)phthalate in Three Sludge-amended New Mexico Soils. J. Environ. Qual. **14** (4), 479-483.

Fawcett DW (1975) Ultrastructure and function of the Sertoli cell. Handbook of Physiology. Ed.-in-chief: John Field. Sect. 7 Endocrinology. Vol 5; Male reproductive system, **2**, 21-56.

Fayz S, Herbert R and Martin AM (1977) The release of plasticizer from polyvinyl chloride haemodialysis tubing. J. Pharm. Pharmac. **29**, 407.

Flaminio LM, Bergia R, De Angelis L, Ferazza M, Marinovich M, Galli G and Galli CL (1988) The fate of leached di-(2-ethylhexyl)-phthalate (DEHP) in patients on chronic haemodialysis. Int. J. Artif. Organs **11**, 428 434.

Fogel S, Findlay M, Scholl C and Warminsky M (1995) Biodegradation of bis(2-ethylhexyl)phthalate in soil. [Pap. Int. In Situ On-Site Bioreclam. Symp.] Biorem. Recalcitrant Org. 3rd, 315-322.

Forshaga (1996) Mjukgöraremission vid tvätt av PVC-golv. Rapport från Forbo-Forshaga 1996-04-30 (in Swedish).

Foster et al. (1982) Toxicol. Appl. Pharmacol. **63**, 120-132.

Foster et al. (2001) Effects of phthalates esters on the developing reproductive tract of male rats, Human Reproduction Update **7**, 231-235.

Foster PM, Turner KJ and Barlow NJ (2002) Antiandrogenic effects of phthalate combination on *in utereo* male reproductive development in the Sprague-Dawley rat: additivity of response? Toxicol. **66** (1-S), 233.

Foster PMD (1999) Personal communication, e-mail of 17 Sep.1999 (re DEHP Review).

Freeman et al. (1981) The effects of Di-(2-ethylhexyl)-phthalate (DEHP) on steroid metabolism in the atlantic cod Gadhus morhua. Proceedings of the seventh annual aquatic toxicity workshop: November 5-7, 1980 Montreal, Quebec.

Freitag D, Geyer H, Kraus A, Viswanathan R, Kotzias D, Attar A, Klein W and Korte F (1982) Screening of chemicals for their environmental behaviour by comparative evaluation. Institut fur Ökologishe Chemie, Gesellschaft f. Stralen- und umweltforschung, Munich, Germany. Ecotoxicology and Environ. Safety **6** (60-81).

Friege H (1986). Dioxin, der Preis des Fortschritts ?, Bund fuer Umwelt- und Naturschutz Deutschland e.V., Freiburg, FRG, zitiert nach: Wams,T.J., The Science of the Total Environment **66**, 1-16.

Fries GF and Marrow GS (1981) Chlorobiphenyl movement from soil to Soybean plants. J. Agric. Food Chem. **29**, 757-799.

Frische R et al. (1979) Bericht des Battelle-Instituts e.V., Frankfurt am Main, an das Umweltbundesamt, Berlin, Forschungsbericht 101 04 009/03.

Fristad J (2000) Alcro Beckers AB Märsta Sweden. Personal communication.

Fujikawa K, Ryo H and Kondo S (1985) The Drosophila reversion assay using the unstable *zeste-white* somatic eye color system. **In**: Progr. Mutat. Res. Ashby J and de Serres FJ (eds), Elsevier Science Publ. **5**, 319-324.

Fukuhara M and Takabatake E (1977) Studies on the liver enlargement induced by dietary administration of di-(2-ethylhexyl) phthalate (DEHP). J. Toxicol. Sci. **2**, 11-23.

Furtmann K (1996) Phalates in the aquatic environment. English translation of the ECPI./CEFIC report 6/93.

Furtmann K (1993) Phthalate in der aquatischen Umwelt, LWA-materialen Nr 6/93, Landesamt für Wasser und Abfall Nordrhein-Westfalen. English translation: ”Phthalates in the aquatic environment”. CEFIC D/1996/3158/4. June 1996.

Gächter R and Müller H (ed.) (1990) Plastic additives Handbook. Hanser Publichers, Munich, Vienna, New York

Gaido KW, McDonnell DP, Korach KS and Safe SH (1997) Estrogenic activity of chemical mixtures: is there synergism? CIIT Activities **17**, 1-7.

Gangolli SD (1982) Testicular effects of phthalate esters. Environ. Health Perspect. **44**, 77-84.

Ganning AE, Brunk U and Dallner G (1984) Phthalate esters and their effect on the liver. Hepatology. **4**, 541-547.

Ganning AE, Brunk U, Edlund C, Elhammer Å and Dallner G (1987) Effects of prolonged administration of phthalate esters on the liver. Environ. Health Perspect. **73**, 251-258.

Ganning AE, Olsson MJ, Brunk U and Dallner G (1990) Effects of prolonged treatment with phthalate ester on rat liver. Pharmacol. Toxicol. **68**, 392-401.

Gans G (1996) Production Process of C8-C10 Phthalates, European Council for Plasticisers and Intermediates, 1996.03.18.

Garner RC and Campbell J (1985) Tests for the induction of mutations to ouabain or 6-thioguanine resistance in mouse lymphoma L5178Y cells. In: Progr. Mutat. Res. Ashby J and de Serres FJ (eds) Elsevier Science Publ. **5**, 525-529,

Garvey LK, Swenberg JA, Hamm Jr,TE and Popp JA (1987) Di-(2-ethylhexyl) phthalate: lack of initiating activity in the liver of female F-344 rats. Carcinogenesis **8**, 285-290.

Gaunt IF and Butterworth KR (1982) Autoradiographic study of orally administered di-(2-ethylhexyl) phthalate in the mouse. Fd. Chem. Toxic. 20, 215-217.

Gejlsbjerg B, Klinge C and Madsen T (2001) Mineralisation of organic contaminants in sludge-soil mixtures. Environ. Toxicol. Chem. **20**, 698-705.

General Electric Co (1976a) TSCATS 206275, Doc. I. D.: 878210884, 08-11-76. The work was carried out by Warf Institute, Inc., Madison, Wisconsin.

General Motors (1982a) Disposition of di(2-ethylhexyl) phthalate following inhalation and peroral exposure in rats. TSCATS: OTS0530339, Doc. I. D.: 86-910000683, 06-22-82, General Motors Corp.

General Motors (1982b) Disposition of di(2-ethylhexyl) phthalate following inhalation and peroral exposure in rats. TSCATS: OTS84003A, Doc. I. D.: 878210878, 07-27-82, General Motors Corp.

General Motors (1982c) Peroxisome proliferation in rats administered di(2-ethylhexyl) phthalate and di(2-ethylhexyl) phthalate-isodecyl phthalate in the diet. TSCATS: 206189, Doc. I. D.: 878210880, 11-19-82, General Motors Corp.

Gerbracht U, Einig C, Oesterle D, Deml E, Schlatterer B and Eigenbrodt E (1990) Di-(2-ethylhexyl) phthalate alters carbohydrate enzyme activities and foci incidence in rat liver. Carcinogenesis **11,** 2111-2115.

Gerstoft J, Christiansen E, Nielsen IL and Nielsen B (1980) The migration of plasticizers from PVC haemodialysis tubes, Proc. Eur. Dial. Transplant. Assoc. **16**, 739-740.

Giam CS et al. (1984) The handbook of the environmental Chem. 3:67-142. Hutzinger O ed. Berlin, Springer-Verlag.

Giam CS, Atlas E, Chan HS and Neff GS (1980) Phthalate esters, PCB and DDT residues in the Gulf of Mexico atmosphere. Atmos. Env. **14**, 65-68.

Giam GS, Chan HS, Neff GS and Atlas EF (1978) Phthalate ester plasticizers: A new class of marine pollutant. Sci. **199**, 419-421.

Gibbons JA and Alexander M (1989) Microbial degradation of sparingly soluble organic chemicals: Phthalate esters. Environ. Toxicol. Chem. **8**, 283-291.

Gibson M (1994). Assessment of fume from plastic packaging film. Toxic Substances Bulletin, No 25.

Gibson TP, Briggs WA and Boone BJ (1976) Delivery of di-2-ethylhexyl phthalate to patients during hemodialysis, J. Lab. Clin. Med. **87**, 519-524.

Gilek M, Björk M, Broman D, Kautsky N, Kausky U and Näf C (1997). The role of the Mussel, *Mytilus edulis*, in the cycling of hydrophobic organic contaminants in the Baltic prober. Ambio **26**, 4.

Gilioli R, Bulgheroni C, Terrana T, Filippini G, Massetto N and Boeri R (1978) Horizontal and longitudinal study of a population employed in the production of phthalates. Med. Lav. **69**, 620-631.

Giust JA Seipelt CT, Anderson BK, Deis DA and Hinders JD (1990) Determination of Bis(2-ethylhexyl)Phthalate in Cow´s Milk and Infant Formulae by High-Performance Liquid Chromatography. J. Agri. Food Chem. **38**, 415-418.

Golder Grundteknik (2000) Översiktliga undersökningar av sediment I Viskans avrinningsområde. Rapport till Länsstyrelsen i Västra Götaland (in Swedish).

Gollamudi R, Prasanna HR, Rao Rh, Lawrence WH and Autian J (1983) Impaired metabolism of di(2-ethylhexyl)phthalate (DEHP) in old rats- an in vitro study. J. Toxicol. Environ. Health **12** (4-6), 623-632.

Gollamudi R et al. (1985) Developmental changes in the conversion rates of di (2-ethylhexyl)phtalate to monoethylhexyl phtalate in rats. J. Toxicol. Environ. Health **15**, 459-465.

Goto M (1979) Monitoring environmental materials and specimen banking: state-of-the-art of Japanese experience and knowledge. **In**: Monitoring environmental materials and specimen banking. Luepka NP (ed), Nijhoff Publ., den Haag.

Graf U, Frei H, Kägi A, Katz AJ and Würgler FE (1989). Thirty compounds tested in the Drosophila wing spot test. Mutation Research **222**, 359-373.

Grant BF (1970) Pesticide effects on fish endocrine functions. Progress in sport Fishery Res., Bur. Sport Fisheries and Wild Life Resources, Canada. **106**, 22.

Grasso P, Heindel JJ, Powell CJ and Reichert Jr LE (1993) Effects of mono(2-ethylhexyl) phthalate, a testicular toxicant, on follicle-stimulating hormone binding to membranes from cultured rat sertoli cells. Biol. Reprod. **48**, 454-459.

Gray LE Jr, Wolf C, Lambright C, Mann P, Price M, Cooper RL and Ostby J (1999) Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, *p,p´*-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ehthane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the rat. Toxicol. Ind. Health **15** (1-2), 94-118.

Gray TJB and Gangolli SD (1986) Aspects of the testicular toxicity of phthalate esters. Environ. Health Perspect. **65**, 229-235.

Gray TJB and Butterworth KR (1980) Testicular atrophy produced by phthalate esters. Arch. Toxicol. Suppl. **4**, 452-455.

Gray TJB, Butterworth KR, Gaunt LE, Grasso P and Gangolli SD (1977) Short-term toxicity study of di(2-ethylhexyl) phthalate in rats. Fd. Cosmet. Toxicol. **15**, 389-399.

Gray TJB, Rowland IR, Foster PMD and Gangolli SD (1982) Species differences in the testicular toxicity of phthalate esters. Toxicol. Lett. **11**, 141-147.

Greener Y, Gillies B, Wienckowski D, Schmitt D, Woods E and Youkilis E (1987). Assessment of the safety of chemicals administered intravenously in the neonatal rat. Teratol. **35**, 187-194,

Greenpeace (1997) Chemical leaching from soft PVC toys. Excerpts from the Greenpeace publication: Children's toys made of plasticized PVC, 1997, summary.

Griffiths WC, Camara PD, Saritelli A and Gentile J (1988) The in vitro serum protein binding characteristics of bis(2-ethylhexyl)phthalate and its principal metabolite, mono-(2-ethylhexyl)phthalate. Environ. Health Perspect. **77**, 151-156.

Gruber L, Wolz G and Piringer O (1998) Untersuchung von Phthalaten in Baby-Nahrung. Deutsche Lebensmittel-Rundschau **9** (6).

Grüttner H et al. (1995) Måleprogram for phthalater på 3 danske renseanlæg. Arbejdsrapport fra Miljøstyrelsen Nr. 54, 1995. Miljøstyrelsen. (in Danish).

Grüttner H and Jacobsen BN (1994) Miljøfremmende stoffer in renseanlæg. Miljøprojekt nr.278. Miljøstyrelsen. (in Danish).

Grüttner H and Vikelsøe J (1996) Miljøfremmende stoffer in spildevand og slam. Miljøprojekt nr. 325. Miljøstyrelsen. (in Danish).

Gulati DK, Sabharwal PS and Shelby MD (1985) Tests for the induction of chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary (CHO) cells. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds) **5**, 413-426.

Gulati DK, Witt K, Anderson B, Zeiger E and Shelby MD (1989) Chromosome aberration and sister chromoatid exchange tests in Chinese hamster ovary cells in vitro III: Results with 27 chemicals. Environ. Mol. Mutagen. **13**, 133-193.

Gunz D, Shephard SE and Lutz WK (1993) Can nongenotoxic carcinogens be detected with the *lacI* transgenic mouse mutation Assay? Environ. Mol. Mutagen. **21**, 209-211.

Gupta RC, Goel SK, Earley K, Singh B and Reddy JK (1985) ^{32}P-Postlabelling analysis of peroxisome proliferator-DNA adduct formation in rat liver *in vivo* and hepatocytes *in vitro*. Carcinogenesis **6**, 933-936.

Häggström J (1997) SVA, Personal communication, march 1997. Original ref. Hall W. C. and Brody S. (1933). Mo. Agr. Exp. Stat. Res. Bull. **180**, 8-31.

Hagiwara A, Diwan BA and Ward JM (1986) Modifying effects of butylated hydroxyanisole, di-(2-ethylhexyl) phthalate or indomethacin on mouse hepatocarcinogenesis initiated by N-nitrosodiethylamine. Jpn. J. Cancer Res. **77**, 1215-1221.

Hagiwara A, Tamano S, Ogiso T, Asakawa E and Fukushima S (1990) Promoting effect of the peroxisome proliferator, clofibrate, but not di(2-ethylhexyl) phthalate, on urinary bladder carcinogenesis in F344 rats initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine. Jpn. J. Cancer Res. **81**, 1232-1238.

Hagmar L, Åkesson B, Nielsen J, Andersson C, Lidén K, Attewell R and Möller T (1990) Mortality and Cancer Mobidity in Workers Exposed to Low levels of Vinyl Chloride Processing Plant. Am. J. Indust. Med. **17**, 553-565.

Hamano Y, Inoue K, Oda Y, Yamamoto H and Kunita N (1979) Studies on the toxicity of phthalic acid esters - dominant lethal tests for DEHP and MEHP in mice. Osaka-furitsu Koshu Eisei Kenkyusho Kenkyu Hokoku. Shokuhin Eisei Hen **10**, 1-4.

Hanberg A, Högberg J, Berglund M, Bensryd I, Skerfving S, Remberger M, Calafat A, Appelgren M, Falk Filipsson A, Jansson B and Håkansson H (2005) Phthalates and their metabolites in human breast milk, blood and urine as measures for monitoring exposure in human risk groups. Report to the Environmental Monitoring programme, Swedish Environmental Protection Agency.

Hanson RL (1983) Inhalation Toxicology Research Institute (ITRI). Phthalate Ester Migration from Polyvinyl Chloride Consumer Products. August 23, Phase 1, Final Report.

Hardell L, Ohlson C-G and Fredrikson M (1997) Occupational exposure to polyvinyl ckloride as a risk factor for testicular cancer evaluated in a case-control study. Int. J. Cancer **73**, 828-830.

Hardin BD et al. (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogenisis, Carcinogenisis and Mutagenesis **7**, 29-48.

Hardwick RC, Cole RA and Fyfield TP (1984) Injury to and death of cabbage (Brassica oleracea) seedlings caused by vapors of di butyl phtalate emitted from certain plastics. Ann. Appl. Biol. **105**, 97-105

Harris CA, Henttu P, Parker MG and Sumpter JP (1997). The estrogenic activity of phthalate esters *in vitro.* Environ. Health. Perspect. **105** (8), 802-811.

Harris RS, Hodge HC, Maynard EA and Blanchet Jr HJ (1956) Chronic oral toxicity of 2-ethylhexyl phthalate in rats and dogs. AMA Arch. Ind. Health **13**, 259-264.

Hartle R (1983) Health Hazard Evaluation report No. HETA-82-127-1370, Hoover Company Industrial Park, North Canton, Ohio. Hazard Evaluations and technical Assistance branch; NIOSH, Cincinnati, Ohio. report No. HETA-82-127-1370, 30 pages, 7 references.

Hatch GG and Anderson TM (1985) Assays for enhanced DNA viral transformation of primary Syrian hamster embryo (SHE) cells. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds) **5**, 629-638.

Hazleton (1983) Screening of priority chemicals for potential reproductive hazard. TSCATS: OTS0536209, New Doc I.D.: 88-920002015, 12-06-83, Centers for desease control, Hazleton Laboratories.

Heitmuller PT, Hollister TA and Parrish PR (1981) Acute toxicity of 54 industrial chemicals to Sheepshead minnow (Cyprinodon variegatus). Bull. Environm. Contam. Toxicol. **27**, 596-604.

Hellwig J, Freudenberger H and Jäckh R (1997) Differential prenatal toxicity of branched phthalate esters in rats. Fd. Chem. Toxicol. **35**, 501-512.

Henderson and Sargent (1983) Sudies on the effects of Di-(2-ethylhexyl)phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed zooplankton rish in wax esters, Comp. biochem. Physiol. **74C** (2), 325-330.

Henderson IS et al. (1987) Factors affecting plasticizer content of unused CAPD fluid. Peritoneal Dialysis Bulletin Suppl. **7** (2), 539.

Henriksson A (1997) Rapport från slamrevision vid Klagshamns reningsverk 1997. Hushållningssällskapet Malmöhus. Borgeby Slott 239 91 Bjäred (in swedish).

Herring R and Bering L (1988) Effects of phthalate esters on plant seedlings and reversal by soil micro-organisms. Bull. Environ. Contam. Toxicol. **40**, 626-632.

Hill EF, Heath RG, Spann JW and Williams JD (1975) Lethal dietary toxicities of environmental pollutants to birds. US Fish anf Wildlife Service, Special Scientific Report – Wildlife No. 191, Washington DC.

Hileman B (2000) Alert On Phthalates. Chem. Engineering News, **78** (32), August 7, 2000.

Hill IR, Matthiessen P and Heimbach F (1994) Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments. From the "Workshop on Sediment Toxicity Assessment" held in the Netherlands, 8-10 November 1993. The book can be ordered from Dr. Ian Hill, ZENECA Agrochemicals, Jealotts Hill Research Station, Braknell, Berkshire RG 12 6YE, UK.

Hillman LS, Goodwin SL and Sherman WR (1975). Identification and measurement of plasticizer in neonatal tissues after umbilical catheters and blood products. The New England J. Med. **292** (8), 381-386.

Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P and Bridges JW (1986) Effects of phthalic acid esters on the liver and thyroid. Environ. Health Perspect. **70**, 195-210.

Hjertberg T (1998). Personal communication. Prof. Inst. of Polymer Technology. Chalmers university of technology, Gothenburg, Sweden.

Hodgson JR (1987) Results of peroxisome induction studies on tri(2-ethylhexyl) trimellitate and 2-ethylhexanol. Toxicol. Ind. Health **3,** 49-60.

HOECHST AG (1980a) unveroeffentlichte Untersuchung (RWL 28.03.80).

HOECHST AG (1980b) ETAD fermentation tube test “Determination of toxic effect against waste water bacteria”. Unpublish report cited in UICLID (not available).

HOECHST AG (1993) Safety data sheet Genomoll 100 (23.05.1993)

Hoenen (2000) Letter from Verband der Keramischen Industrie E.V. Germany about “Softener in the ceramic body”. 13 july 2000.

Hoenich NA, Thompson J, Varini E, McCabe J and Appleton D (1990) Particle spallation and plasticizer (DEHP) release from extracorporeal circuit tubing matenals Int. J. Artif. Organs **13**, 55-62.

Holmes RS and Masters CJ (1967) The developmental multiplicity and isoenzyme status of rat esterases. Biochem Biophys Acta. **146** (1), 138-150.

Horowitz B, Stryker MH, Waldmann AA, Woods KR, Gass JD and Drago J (1985) Stabilisation of red blood cells by the plasticiser diethylhexylphthalte. Vox. Sang. **48**, 150-155.

Howard PH et al. (1985) Measurement of Water Solubilities, Octanol/Water Partition Coefficients and Vapor Pressures of Commercial Phthalate Esters, Environ. Toxicol. Chem. **4**, 653.

Howard PH (1989) Fate and Exposure data.

Hrudey SE, Sergy GA and Thackeray T (1976) Toxicity of oil sands plant wastewaters and associated organic contaminants. Proc. 11th canadian symp. 1976: Water pollution research Canada.

HSE (Health and Safety Executive) (1984) Advisory Committee on Toxic Substances document ACTS 21/85 Annex 1 ”Phthalate esters: Production. usage, and occupational exposure”. Health and Safety Executive, September 1984. See also: King DA (1996). Personal communication: “Occupational exposure to phthalate esters with specific reference to Di-Ethyl Hexyle Phthalate (DEHP), Di-Butyl Phthalate (DBP), Di-Isononyl Phthalate (DINP), and Di-Isodecyl Phthalate (DIDP)”. BP Chemicals Ltd., Hull, UK.

HSDB (Hazardous Substances Data Bank) search on CAS no 117-81-7, done 2006-02-28; http://toxnet.nlm.nih.gov

Huber WW, Grasl-Kraup B and Schulte-Hermann R (1996) Hepatocarcinogenic Potential of Di(2-ethylhexyl)phthalate in rodents and its implications on human risk. Critical Reviews in Toxicol. **26** (4), 365-480.

Hüls (1981) Safety tests on Vestinol AH di-2-ethylhexylphthalate. IRI Project No. 416725.

Hüls (1987a) Prüfung der akuten Hautreizwirkung von VESTINOL AH. Bericht Nr. 1071.

Hüls (1987b) Prüfung der akuten Augen- und Schleimhautreizwirkung von VESTINOL AH. Bericht Nr. 1072.

Hüls AG (1994) Bestimmung der biologischen Abbaubarkeit von VESTINOL AH im Modifizierten Sturm-Test, Abschlussbericht ST-89/94, Hüls, 1994.12.06. (unpublished).

Hüls AG (1995a) Determination of the acute effects of VESTINOL AH on fish, Hüls, 1995.01.25.

Hüls AG (1995b) Determination of the effects of VESTINOL AH on the swimming behaviour of Daphnia magna, Final report DK-631, Hüls, 1995.01.24.

Hüls AG (1995c) Determination of the effects of VESTINOL AH on the reproduction rate of Daphnia magna (as specified by 92/69 EEC, Dec. 1992), Final report DL-160, Hüls, 1995.01.12.

Hüls AG (1995d) Determination of the effects of VESTINOL AH on the growth of Scenedesmus subspicatus 86.81. SAG, Final report AW-391 Hüls, 1995.01.19

Hüls AG (1995e) Bericht über die Messungen on Stoffen in der Luft in Arbeitsbereichen gemäss TRGS 402, (Report on occupational exposure to DEHP and DINP. Monitoring data from application of car underseal). Hüls, 1995.06.07

Hüls AG (1995f) Determination of the effect of Vestinol AH. Final report AW-391. 17 ppg.

Hüls AG (1995g) Analysen-befund., Dr. Schneider. FB FZ / Analytik 1. Unpublished report Marl (1995.05.24)..

Hüls AG (1996) Bestimmung der bakterientoxizität von Vestinol AH, Di-2-ethylhexylphthalate. Report No. ABSK-VEST AH. 11 ppg, Unpublished report.

Hüls AG (1997) Dampfdruck von Di-2-Ethylphtalat (DOP). Unpublished report (15.01.1997).

Hüls AG (1996) Exposure of Bis(2-ethylhexyl)phthalate at Hüls AG, Marl.

Hüls AG (1997) Personal communication with B. Mentzel, Hüls, 16th Januari 1997.

HülsInfracor (1999) Vestinol AH. Determination of the inhibition of activated sludge respiration. Final report BH-99/01. 22 ppg. (unpublished)

Huntingdon (1997) Phthalic acid, di(2-ethylhexyl) ester (DEHP): Study of embryo-foetal toxicity in the CD-1 mouse by oral gavage administration. Huntingdon, Report no 95/EHM007/0705.

Hutchins et al. (1985) in TSD 1991.

IARC (1995) International Agency for Research on Cancer. Peroxisome Proliferation and its role in Carcinogenesis, views and expert opinions of an IARC Working Group Lyon, 7-11 Dec 1994, IARC Technical Report No.24 Lyon.

IARC (International Agency for Research on Cancer) (1996) Clofibrate. Gemfibrozil. In: IARC monographs on the evaluation of carcinogenic risks to humans, some pharmaceutical drugs, vol. 66, Lyon.

ICI (1982a) Bis(2-ethylhexyl) phthalate: Toxicokinetics of 14-day subacute oral administration to rats and marmosets. TSCATS: 215194, Doc. I. D.: 878220041, 06-02-82, ICI Americas Inc.

ICI (1982b). Di(2-ethylhexyl) phthalate: a comparative subacute toxicity study in the rat and marmoset. TSCATS: 215194, Doc. I. D.: 87-8220040, 05-11-82, ICI Americas Inc.

ICI (1984) PLC; Assessment of the biodegradability of Di-2-ethyl-hexylphthalate (DEHP) and Di-isodecylphthalate (DIDP) by a modification of OECD Test Guideline 301 C, Report No. BLS/B/0240.

ICPS/WHO (1992) International Programme on Chemical Safety/World Health Organization, Dihexyl phthalate. Environ. Health Criteria **131**, 1-141.

Ikeda GJ, Sapienza PP, Couvillion JL, Farber TM and van Loon EJ (1980) Comparative distribution, excretion and metabolism of di-(2-ethylhexyl) phthalate in rats, dogs and miniature pigs. Fd. Cosmet. Toxicol. **18**, 637-642.

INDUSTRY (1999) Risk assessment Bis(2-ethylhexyl)phthalate, CAS No 117-81-7. Update of Emission Data. Letter from K. Schwarz. 1999.09.06.

INDUSTRY (1999b) "Formulation and processing sites" Letter fromK. Schwarz to KemI. 10 Nov 1999.

INDUSTRY (2000) Appendix 5 in a Letter from K. Schwarz to KemI January 2000.

Inge-Vechtomov SG, Pavlov YI, Noskov VN, Repnevskaya MV, Karpova TS, Khromov-Borisov NN, Chekuolene J and Chitavichus D (1985). Tests for genetic activity in the yeast *Saccharomyces cerevisiae*: study of forward and reverse mutation, mitotic recombination and illegitimate mating induction. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds). **5**, 243-255

INRS (1997) COLCHIC national database query from 1987. Occupational exposure to phthalates. (Ref in Risk Assessment Report 2/9/991,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters C9-rich and Di-"isononyl"phthalate).

Institut für Wasser-, Boden u Lufthygiene, Umweltsbundesamt (1995) Ökotoxizität von Di-(2-etylhexyl)-phthalate, Akuter Daphnientest (24h/48/) mit Daphnia magna nach DIN 38412 Teil II, Berlin,1995.09.11

Irvine RL and Cassidy DP (1995) Periodically operated bioreactors for the treatment of soila and leachates. Biol. Unit Processes Harzard. Pap. Int. Situ On-site bioreclam. 3rd Symp 1995. 289-298.

Ishida M, Suyama K and Adachi S (1981). J. Agric. Food Chem. **29**, 72-74.

Ishida M, Suyama K and Adachi S (1982) Distribution of orally administered diethylhexyl phthalate in laying hens. Poult. Sci. **61**, 262-267.

Ishidate Jr M and Sofuni T (1985) The in vitro chromosomal aberrration test using Chinese hamster lung (CHL) fibroblast cells in culture. **In**: Progr. Mutat. Res., Elsevier Science Publ. Ashby J and de Serres FJ (eds). **5**, 427-432.

Ito N, Tsuda H, Tatematsu M, Inoue T, Tagawa Y, Aoki T, Uwagawa S, Kagawa M, Ogiso T, Masui T, Imaida K, Fukushima S and Asamot M (1988) Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats - an approach for a new medium-term bioassay system. Carcinogenesis **9**, 387-394.

IUCLID (1996) Source: BASF AG. Creation date: 02-SEP-93; printing date: 15-APR-96; revision date: 20-MAR-96; date of last Update: 20-MAR-96.

Jaakkola JJ, Oie L, Nafstad P, Botten G, Samuelsen SO and Magnus P (1999) Interior surface materials in the home and the development of bronchial obstruction in young children in Oslo, Norway. Am. J. Pub. Health. **89** (2), 118-192.

Jacobs G and Moffid A (1988) Abbau und Anreichung ausgewälter persistenter Stoffe: Phthalsäureester. Vorkommen und Verteilung von Phthalsäureestern in der Elbe – Untersuchungen im Süsswasserwatt Heuckenlock und im Harburger Hafen. Bericht der Arbeitsgemeinschaft zur Förderung angewandter biologischer Forschung e.V., Hamburg, an das Umweltbundesamt, Berlin. Berich Nr. 106 07 054, Hamburg.

Jacobson MS, Kevy SV and Grand RJ (1977) Effects of a plasticizer leached from polyvinyl chloride on the subhuman primate: a consequence of chronic transfusion therapy. J. Lab. Clin. Med. **89**, 1066-1079.

Jaeger RJ and Rubin RJ (1970) Contamination of blood stored in plastic packs. Lancet. **2**, 151.

Jaeger RJ and Rubin RJ (1972) Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues. The New England J. Med. **287**, 1114-1118.

Jaeger RJ and Rubin RJ (1973) Extraction, localization, and metabolism of di-2- ethylhexyl phthalate from PVC plastic medical devices. DHEW Publication. IVIH 73-318, Environ. Health. Persp. **3**, 95-102.

Jain GC and Joshi SC (1991) Effects of plasticizer di(2-ethylhexyl) phthalate (DEHP) on reproductive function of mice. Z. Ange. Zool. **78**, 465-470.

James NH, Soames AR and Roberts RA (1998) Suppression of hepatocyte apoptosis and induction of DNA synthesis by the rat and mouse hepatocarcinogen diethylhexylphthalate (DEHP) and the mouse hepatocarcinogen 1,4-dichlorobenzene (DCB). Arch. Toxicol. **72**, 784-790.

Jarosová A, Gajdusková V, Raszyk J and Sevela K (1999) Di-2-ethylhexyl phthalate and di-n-butyl phthalate in the tissues of pigs and broiler chicks after their oral administration. Vet. Med.-Czech. **44**, 61-70

Jensen J, van Langevelde J, Pritzl G and Krogh PH (2001) Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan *Folsomia fimetaria*. Environ. Toxicol. Chem. **20**, (5), 1085-1091.

Jobling S, Reynolds T, White R, Parker MG and Sumpter JP (1995) A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ. Health Perspect. **103**, 582-587.

Johnson BT, Heitkamp MA and Jones JR (1984) Environmental and chemical factors influencing the biodegradation of phthalic acid esters in freshwater sediments. Environ. Pollut. Ser. B. **8** (2), 101-118.

Johnson B and Lulves W (1975) Biodegradation of di-n-butyl phthalate and di-2-ethylhexyl phthalate in freshwater hydrosoil. US Dept. of Interior, Columbia, USA. Journ. of the Fisheries Research Board of Canada. **32** (3), 333–339.

Johnson WW and Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Dept. of the Interior, Fish and Wildlife Service, Res. Pub. **137**, 65.

Jones CA, Huberman E, Callaham MF, Tu A, Halloween W, Pallota S, Sivak A, Lubet RA, Avery MD, Kouri RE, Spalding J and Tennant RW (1988) An interlaboratory evaluation of the Syrian hamster embryo cell transformation assay using eighteen coded chemicals. Toxic. in Vitro **2**,103-116.

Jones HB, Garside DA, Liu R and Roberts JC (1993) The influence of phthalate esters on Leydig cell structure and function *in vitro* and *in vivo*. Exp. Mol. Pathol. **58**, 179-193.

Joslin MT (1996) Environmental assessment of Di-2-Ethyl Hexyl Phthalate (DEHP) production at BP Chemicals Ltd Hull Site

Jung R, Engelhart G, Herbolt B, Jäckh R and Müller W (1992). Collaborative study of mutagenicity with *Salmonella typhimurium* TA 102. Mut. Res. **278**, 265-270.

Källqvist T (1991). Karakterisering av avloppsvatten från neste Oxo AB Stenungsund, kompletterad rapport. Norsk Institutt for vannforskning. NIVA rapport O-90114. ISBN 82-577-1913-7 (in Swedish an Norwegian).

Kamata I, Tsutsui G, Takana J and Shirai T (1978) Phthalic acid esters in fish in the Uji river. Kyoto-fu Esei Kogai Kenkyosho Nempo **22**, 114.

Kampe W (1987) Korrespondenz Abwasser 34, 820-827, (1987), zitiert nach Rippen, Handbuch Umweltchemikalien, 13.Erg.Lfg.1/92

Kampe W et al. (1986) Schadstoffe im Boden, insbes. Schwermetalle und organische Schadstoffe aus langjaehriger Anwendung von Siedlungsabfaellen, Bericht der Landwirtschaftlichen Untersuchungs- und Forschungsanstalt Speyer an das UBA Berlin, zitiert nach: Rippen, Handbuch Umweltchemikalien, 13.Erg.Lfg.1/92

Karara AH and Hayton WL (1984) Pharmacokinetic model for the uptake and disposition of Di-2-ethylhexyl Phthalate in Sheepshead minnow. College of Pharmacy, Washington state univ., Pullman, Washington. Aquatic Toxicology **5**, 181-195.

Kasai H, Okada S, Nishimura, Rao MS and Reddy JK (1989) Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term expopsure to a peroxisome proliferator. Cancer Res. **49**, 2603-2605.

Kaufmann G et al. (1992) Battelle-Institut, Frankfurt am Main, unveroeffentlicht, zitiert nach: Rippen, Handbuch Umweltchemikalien, 13.Erg.Lfg.1/92.

Kaul AF, Souney PF and Osathannondh RA (1982) A review of possible toxicity of di-2-ethylhexylphthalate (DEHP) in plastic intravenous containers: effects on reproduction. Drug Intell. Clin. Pharm. **16**, 689-692.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Earl Gray L, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka, C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T and Tilson HA (1996) Research needs for the risk assessment of health and environmental effects of endocrine disrupters: a report of the U.S. EPA-sponsored workshop. Environ. Health Perspect. **104**, 715-740.

KemI (1997) Additives in PVC, Marketing of PVC. Report No. 4/97. The Swedish National Inspectorate.

KEMI (1997) Hormonella effekter av kemikalier - en samman fattning av kunskapsläget. Rapport från Kemikalieinspektionen 1/97.

Kenrick et al. (1985) in TSD 1991.

Kevy SV and Jacobson MS (1982) Hepatic effects of a phthalate ester plasticizer leached from poly(vinyl chloride) blood bags follwoing transfusion. Environ. Health Perspect. **45**, 57-64.

Khaliq MA and Srivastava SP (1993) Induction of hepatic polyamines by di(2-ethylhexyl) phthalate in rats. Toxicol. Letters **66**, 317-321.

Kice JL and Marvell EN (1974) Modern principles of Organic Chemistry.ISBN: 0-02-362890-2. Macmillan Publishing Co., Inc.

Kim E-J, Kim J-W and Lee S-K (2002) Inhibition of oocyte development in Japanese medaka (*Oryzias latipes*) exposed to di-2-ethylhexyl phthalate. Environ. Int. **28**, 359-365.

King DA (1996) Personal communication: ”Occupational Exposure to Phthalate Esters with specific reference to Di-ethyl Hexyle Phthalate (DEHP), Di-Butyl Phthalate (DBP), Di-isononyl Phthalate (DINP) and Di-Isodecyl Phthalate (DIDP)”. BP Chemicals Ltd., Hull, UK.

Kirby PE, Pizzarello RF, Lawlor TE, Haworth SR and Hodgson JR (1983) Evaluation of di(2-ethylhexyl) phthalate and its major metabolites in the Ames test and L5178Y mouse lymphoma mutagenicity assay. Environ. Mutagen. **5**, 657-663.

Kirchmann H and Tengsved A (1991) Organic Pollutants in sewage sludge: 2. Analysis of barley grains grown on sludge-fertilized soil. Swedish J. Agric. Res. **21**, 115-119.

Kirchmann H, Åström H and Jönsäll G (1991) Organic Pollutants in Sewage Sludge 1. Swedish J. Agric. Res. **21**, 107-113.

Kjølholt J, Thomsen CD and Hansen E (1998) Cadmium og DEHP in kompost og bioafgasset materiale. Miljöprojekt nr. 385. Miljöstyrelsen, Denmark.

Kjølholt J, Andersen HV and Poll C (1995) Forekomst og effekter af miljøfremmede organiske stoffer in spildevandslam. Arbejtsrapport fra Miljøstyrelsen. Nr. 15, 1995. Denmark.

Klaassen, Amdur and Doull (1986) Toxicology, Fourth Edition. The basic Science of Poision.

Klaunig JE et al. (2003) PPARα Agonist-Induced Rodent Tumors: Modes of Action and Human Relevance. Crit. Rev. Toxicol. **33** (6), 655-780

Klein W, Kördel W, Weiß M and Poremski HJ (1988) Chemosphere **17** (2), 361-386.

Klimisch H-J, Gamer AO, Hellwig J, Kaufmann W and Jäckh R (1992) Di(2-ethylhexyl) phthalate (DEHP): A short-term repeated inhalation toxicity study including fertility assessment. Fd. Chem. Toxic. **30**, 915-919.

Klöpffer W, Kaufmann G, Rippen G and Poremski H-J (1982) Ecotox., Environ. Saf. **6**, 545-559.

Klöpffer W and Kohl EG (1990) Determination of the Rate Constant k(OH)(air) Using Freon 113 as an Inert Solvent, Paper presented at the 3rd Workshop on Study and Prediction of Pesticides Behaviour in Soils, Plants and aquatic Systems, Muenchen-Neuherberg; (30.5.-1.6.1990).

Kluwe WM, Haseman JK and Huff JE (1983) The carcinogenicity of di-(2-ethylhexyl) phthalate (DEHP) in perspective. J. Toxicol. Environ. Health **12**, 159-169.

Kluwe WM, Haseman JK, Douglas JF and Huff JE (1982) The carcinogenicity of dietary di-(2-ethylhexyl) phthalate (DEHP) in Fischer 344 rats and B6C3F1 mice. J. Toxicol. Environ. Health **10**, 797-815.

Knowles CO, Mckee MJ and Palawski DU (1987) Chronic effects of di-2-ethylhexyl phthalate on biochemical composition, survival and reproduction of Daphnia magna. Environ. Toxicol. Chem. **6**, 201-208.

Koch M, Drexler H and Angerer J (2003a) An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. Int. J. Environ. Health **206**, 77-83.

Koch HM, Gonzales-Reche LM and J Angerer (2003b) On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. J. Chromatogr. B Analyt. Technol. Biomed .Life Sci. **784** (1), 169-82.

Koch HM, Rossbach B, Drexler H and Angerer J (2003c). Internal exposure of the general population to DEHP and other phthalates—determination of secondary and primary phthalate monoester metabolites in urine. Environ. Res. **93**, 177-185.

Koch HM, Bolt HM and Angerer J (2003d) Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol, published on line 24 October 2003. DOI: 10.1007/s00204-003-0522-3.

Koch HM, Drexler H and Angerer J (2004) Internal Exposure of Nursery-School Children and their Parents and Teachers to Di(2-ethylhexyl)phthalate (DEHP). Int. J. Hyg. Environ. Health **207**, 15-22.

Koch R (1991) Umweltchemikalien, 2.Auflage, VCH Weinheim.

Kodama T and Takai Y (1974) Studies on the deetermination of phthalic acid esters, IV. Analysis of phthalic acid esters in fish meat. Kogai To Taisaku **10**, 850.

Kolb M, Welte K, Mettenleiter and Trinkmann A (1997). Bestimmung von Phthalsäureestern in Klärschlämmen mittels GC/MS. Wasser and Boden **49** (5), 57-60.

Kömp P and McLachlan MS (1997) Influence of temperature on the Plant/Air partitioning of semivolatile organic compounds. Environ. Sci. Technol. **31**, 886-890.

Könemann WH (ed.). Phthalate rerlease from soft PVC baby toys. Report from the Dutch Consensus Group. (1998). RIVM-national Institute of Public Health and the Environment.

Kornbrust DJ, Barfknecht TR and Ingram P (1984) Effect of di(2-ethylhexyl) phthalate on DNA repair and lipid peroxidation in rat hepatocytes and on metabolic cooperation in Chinese hamster V-79 cells. J. Toxicol. Environ. Health **13**, 99-116.

Kozumbo WJ, Kroll R and Rubin RJ (1982) Assessment of the mutagenicity of phthalate esters. Environ. Health Perspect. **45**, 103-109.

Kristensen P et al. (1996) Anvendelse af affaldsproducter till jordbrugsformål. Miljöprojekt 328. (in Danish)

Krogh PH et al. (1996) Økologisk vurdering af spildevndsslam in landbrugsjord. Arbejdsrapport fra Miljøstyrelsen Nr. 43, 1996. Miljøstyrelsen. (in Danish).

Kroschwitz JI (1998) Kirk-Othmer Encyclopedia of Chemical Technology. Fourth Edition. John Wiley and Sons, New York.

Kubota Y (1979) Experience with the chemical substances control law in Japan. Ecotox. Environ. Safety **3**, 256.

Kurane R, Suzuki T and Takahara Y (1980) Induction of enzymes involved in phthalate esters metabolisms in Nocardia erythropolis and enzymatic hydrolysis of phthalate esters bo commersial lipases. Agric. Biol. Chem. **44**, 529-536.

Kurata Y et al. (1996) 13-week repeated -–dose oral toxicity study of Di-(2-ethylhexyl)phthalate (DEHP) in common marmoset. Presented at SOT Cincinnati, March 1997.

Kurata Y, Kidate F, Tsuchiya M, Yokoyama M, Toyoda N and Takano K (1995) Thirteen week repetive oral administration test of di(2-ethylhexyl)phthalate using marmosets. Test number 4L209. Mitsubishi Chemical Co., Safety Science Laboratories.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Takano K, Tsuchitani M and Katho M (1996) 13-week repeated-dose oral toxicity study of di(2-ethylhexyl) phthalate (DEHP) in common marmoset. Mitsubishi Chemical Safty Institute, preliminary report 1996.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M and Katoh M (1998) Subchronic toxicity of di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicol. Sci. **42,** 49-56.

Kurokawa Y, Takamura N, Matushima Y, Imazawa T and Hayashi Y (1988) Promoting effect of peroxisome proliferators in two-stage rat renal tumorigenesis. Cancer Lett. **43**, 145-149.

Kylin H (1997) Pesonal comunication. Ultuna Agriculture University Sweden.

Labow RS, Card RT and Rock G (1987) The effect of the plasticizer di(2-ethylhexyl) phthalate on red cell deformability Blood **70**, 319-323.

Labow RS, Tocchi M and Rock G (1986) Effects of leachable materials on morphology and function, Transfusion **26**, 351 -357.

Lake BG, Gangolli SD, Grasso P and Lloyd AG (1975) Studies on the hepatic effects of orally administered di-(2-ethylhexyl) phthalate in the rat. Toxicol. Appl. Pharmacol. **32**, 355-367.

Lake BG, Brantom PG, Gangolli SD, Butterworth KR and Grasso P (1976) Studies on the ffects of orally administrated di-(2-ethylhexyl)phthalate in the ferret. Toxicol. **6** (3), 341-356.

Lake BG, Phillips JC, Linnell PJ and Gangolli SD (1977) The *in vitro* hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. Toxicol. Appl. Pharmacol. **39**, 239-248.

Lake BG, Rijcken RP, Gray TJB, Foster JR and Gangolli SD (1984a) Comparative studies of the hepatic effects of di- and mono-n-octyl phthalates, di(2-ethylhexyl) phthalate and clofibrate in the rat. Acta. Pharmacol. Toxicol. **54**, 167-176.

Lake BG, Gray TJB, Foster JR, Stubberfield CR and Gangolli SD (1984b) Comparative studies on di-(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation in the rat and hamster. Toxicol. Appl. Pharmacol. **72**, 46-60.

Lake BG, Kozlen SL, Evans JG, Gray TJB, Young PJ and Gangolli SD (1987) Effect of prolonged administration of clofibric acid and di(2-ethylhexyl) phthalate on hepatic enzyme activities and lipid peroxidation in the rat. Toxicol. **44**, 213-228.

Lamb IV JC, Chapin RE, Teague J, Lawton AD and Reel JR (1987) Reproductive effects of four phthalic acid esters in the mouse. Toxicol. Appl. Pharmacol. **88**, 255-269.

Langerbartels C and Harms H (1986) Plant cell suspension cultures as test systems for an ecotoxicologic evaluation of chemicals -– growth inhibition effects and comparison with the metabolic fate in intact plants. Angew. Botanik **60**, 113-123.

Larose L and Morisset J (1977) Acinar cell responsiveness to urecholine in the rat pancreas during fetal and early postnatal growth. Gastroenterol. **73** (3), 530-3.

Larsson Per. Personal communication. Dept. of Chemical Ecology and Ecotoxicology, University of Lund, Sweden.

Larsson P and Thurén A (1987) Di-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bvioaccumulated by tadpoles. Environm. Toxicol. Chem. **6**, 417-422.

Larsson P, Thurén A and Gahnström G (1986) Phatalate esters inhibit microbial activity in aquatic sediments. Environ. Poll. (Series A). **42**, 223-231.

Laskey JW and Berman E (1993) Steroidogenic assessment using ovary culture in cycling rats: Effects of bis(2-diethylhexyl) phthalate on ovarian steroid production. Reprod. Toxicol. **7**, 25-33.

Laughlin RB and Neff JM (1978) The effects of three phthalate esters on the larval development of the grass shrimp Palamonetes pugio (Holthuis). Water Air and Soil Poll. **9**, 323-336.

Lawrence N and McGregor DB (1985) Assays for the induction of morphological transformation in C3H/10T½ cells in culture with and without S9-mediated metabolic activation. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds) **5**, 651-658.

Lawrence WH, Malik M and Autian J (1974) Development of a toxicity evaluation program for dental materials and products. II. Screening for systemic toxicity. J. Biomed. Mater. Res. **8**, 11-34.

Lawrence WH, Malik M, Turner JE, Singh AR and Autian J (1975) A toxicological investigation of some acute, short-term, and chronic effects of administering di(2-ethylhexyl) phthalate (DEHP) and other phthalate esters. Environ. Res. **9**, 1-11.

LeBlanc GA (1980) Acute toxicity of priority pollutants to water flea (Daphnia magna). Bull. Environm. Contam. Toxicol. **24**, 684-691.

Ledwith BJ, Manam S, Troilo P, Joslyn DJ, Galloway SM and Nichols WW (1993) Activation of immediate-early gene expression by peroxisome proliferators in vitro. Molec. Carcinogen. **8**, 20-27.

Lee S-T, Pineau T, Drago J et al. (1995) Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cellular Biol. **15**, 3012-3022.

Legrand P (1996) Plasticizer Consumption and End-Use Patterns in Western Europe, European Council for Plasticisers and Intermediates, 1996.03.18.

Leibold E, Greim H and Schwarz LR (1994) Inhibition of intercellular communication of rat hepatocytes by nafenopin: involvement of protein kinase C. Carcinogenesis **15**, 1265-1269

Lemberger T, Saladin R, Vasquez M, Assimacopoulos F, Staels B, Desvergne B, Wahli W and Auwerk J (1996). Expression of the peroxisome proliferator-activated receptorα gene is stimulated by stress and follows a diurnal rhythm. J. Biol. Chem. **271**, 1764-1769.

Lemberger T, Staels B, Saladin R, Desvergne B, Auwerk J and Wahli W (1994) Regulation of the peroxisome proliferator-activated receptorα gene by glucocorticoids. J. Biol. Chem. **269**, 24527-24530.

Letter to D. King from D. Cadogan. Appendixes "properties and uses of a number of phthalate esters, level of occupational exposure and hazards associated with exposure".

Levnedsmiddelstyrelsen (1996) Phthalater i modersmælkerstatninger. LST-Nyt 04/96.

Lewis LM, Flechtner TW, Kerkay J, Pearson KH and Nakamoto S (1978). Bis(2-ethylhexyl) phthalate concentration in the serum of hemodialysis patients. Clin. Chem. **24** (5), 741-746.

Leyder F and Boulanger P (1983) Ultraviolet Absorption, Aqueous Solubility, and Octanol-Water Partition for Several Phthalates, Bull. Environ. Dontam. Taoicol. **30**, 152-157.

LGC (1998) Consumer safety Research report -Laboratory-Based Agitation methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare Articles. LGC technical Report Number: LGC/1998/DTI/009. september 1998.

Lhuguenot J-C, Mitchell AM, Milner G, Lock EA and Elcombe CR (1985) The metabolism of di(2-ethylhexyl phthalate (DEHP) and mono(2-ethylhexyl) phthalate (MEHP) in rats: *In vivo* and *in vitro* dose and time dependency of metabolism. Toxicol. Appl. Pharmacol. **80**, 11-22.

Li LH, Jester WF, Laslett AL and Orth Jm (2000) A single dose of di-(2-ethylhexyl) phthalate in neonatal rats alters gonocytes, reduces Sertoli cell proliferation, and decreases cyclin D2 expression. Toxicol. Appl. Pharmacol. **166**, 222-229

Li L-H, Jester WF Jr and Orth JM (1998) Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. Toxicol. Appl. Pharmacol. **153** 258-265.

Liber HL (1985) Mutation tests with Salmonella using 8-azaguanine resistance as the genetic marker. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds). **5**, 213-216.

Ligocki MP, Leuenberger C and Pankow JF (1985) Trace organic compounds in rain part II. Atmos. Environ. **19** (10), 1609-1617.

Lillesand TM and Kiefer R (1979) Remote sesing and image interpretation. J. Wiley and sons, new york. ISBN 0-471-08211-2.

Lillienberg L and Dahlman-Höglund A (1999) Mätning av dietylhexylftalat (DEHP) vid mjukmedelsanläggningen vid neste Oxo i Stenungsund 1999-01-12 och 1999-01-14. Sahlgrenska Univesitetssjukhuset, Sahlgrenska, Yrkesmedicin.

Lindén E, Bengtsson B-E, Svanberg O and Sundström G (1979) The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (Alburnus alburnus) and the harpactoid Nitocra spinipes. Chemosphere Nos 11/12, pp 843-851.

Lindgren A, Lindquist NG, Lydén A, Olsson T and Ullberg S (1982) A whole body autoradiographic study on the distribution of ^{14}C-labelled di(2-ethylhexyl) phthalate in mice. Toxicol. **23**, 149-158.

Lington AW, Bird MG, Plutnick RT, Stubblefield WA and Scala RA (1997) Chronic toxicity and carcinogenic evaluation of Diisononyl phthalate in rats. Fundam. Appl. Toxicol. **36**, 79-89.

Lington AW, Chin EE, Short RD, Astill BD, Moran EJ and Robinson E (1987) Oral disposition and metabolism studies on DEHP: single and repeated dietary dosis in F-344 rats. Unpublished data, 09/02/1987.

Liss GM, Albro PW, Hartle RW and Stringer WT (1985) Urine phthalate determinations as an index of occupational exposure to phthalic anhydride and di-(2-ethylhexyl) phthalate. Scand. J. Work Environ. Health **11**, 381-387.

Little AD (1982) Phthalates in consumer products. Contr. CPSC-C-80-1001. Consumer Product Safety Commission.

Loftus NJ, Woollen BH, Steel GT, Wilks MF and Castle L (1994) An assessment of the dietary uptake of Di-2-(ethylhexyl)adipate (DEHA) in a limited population study. Fd.Chem.Toxic. **32** (1), 1-5.

Lökke H (1988) **In**: Kemiske stoffer in landjordmiljöer. Ed: Helweg A. Forlag A/S, Copenhagen. In Danish.

Lökke H and Bro-Rasmussen L (1983) Phytotoxicological effects of di-(2-ethylhexyl)-phthalate and di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution (Series A). **32**, 179-199.

Lökke H and Bro-Rasmussen F (1981) Studies of mobility of di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere **10**, 1223-1235.

Loman Jon. Personal communication. Dept. of Animal Ecology, Lund University, Sweden

Long JLA, House WA, Parker A and Rae JE (1998) Micro-organic compounds associated with sediments in the Humber rivers. The Science of the Total Environment **210/211**, 229-253.

Loprieno N, Boncristiani G, Forster R and Goldstein B (1985) Assays for forward mutation in *Schizosaccharomyces pombe* strain P1. **In**: Progr. Mutat. Res. Elsevier Science Publ. Ashby J and de Serres FJ (eds). **5**, 297-306.

Lovekamp and Davis (2001) Toxicology and Applied Pharmacology, Mono-(2-ethylhexyl)phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. **172**, 217-224

Lowenbach WA, Golden R, Liberson G and Reese E (1995) A model for estimating beef and milk bioconcentration factors for DEHP Report from Environmental Risk Sciences Ins.

Lundberg G and Nilsson C (1994) Phthalic Acid Esters Used as Plastic Additives. Report No. 12/94. ISSN: 0284-1185 . The Swedish National Chemicals Inspectorate.

Lundberg P, Löf A, Johansson G, Wenberg A, Högberg J and Holmberg B (1991) New Swedish Occupational Standars for Some Organic Solvents. Am. J of Ind. Med. **19**, 559-567.

Lyman WJ et al. (1989) zitiert nach: Howard,P.H., Fate and exposure data. 279.

Maag J and Lökke H (1991) Land treatment of DEHP-contaminated soils.; Atlastensanirung 90, Int. KfK/TNO Kongr,. 3rd, Meeting Date 1990, 1079-1087. Ed. by: Arendt, Friedrich Hisenveld, M., Van den Brink, W.J. Bundesminist. Forsch. Technol.: Bonn, Germany.)

MabeyWR et al. (1982) EPA Report No.440/4-81-014 by SRI Inter- national, Menlo Park, CA, USA, zitiert nach: Rippen, Handbuch Umweltchemikalien, 13. Erg.Lfg. 1/92

Mackintosh CE, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou MG and Gobas FAPC (2004) Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. Environ. Sci. Technol. **38**, 2011-2020.

Madsen PL et al. (1999) Kinetics of Di-(2-ethylhexyl)phthalate mineralisation in sludge-amended soil. Environ. Sci. Technol. **33**, 2601-2606.

MAFF (1990) Ministry of Agriculture, Fisheries and Food. Plasticisers: Continuing surveillance. Food Surveillance Papers No 30.

MAFF (1995) Ministry of Agriculture, Fisheries and Food. Phthalates in paper and board packaging. Food Surveillance Information Sheet 60.

MAFF (1996a) (Ministry of Agriculture, Fisheries and Food). Phthalates in food, Food surveillance information sheet, No 82, March 1996.

MAFF (1996b) Phthalates in infant formulae, Food surveillance information sheet, No 83, March 1996.

MAFF (1996c) Ministry of Agriculture, Fisheries and Food. Survey of plasticiser levels in food contact materials and in foods. Food Surveillance Papers No 21. 1996.

MAFF (1998a) Ministry of Agriculture, Fisheries and Food. Food surveillance information sheet N°168. « Phthalates in infant formulae – Follow-up survey

MAFF (1998b) Ministry of Agriculture, Fisheries and Food: National Food Survey 1998.

MAFF (1998c) Ministry of Agriculture, Fisheries and Food. R&D and Surveillance Report:410.

MAFF (1998d) Ministry of Agriculture, Fisheries and Food. Annual Report on Food Expenditure, Consumption and Nutrient Intakes

MAFF (2000) Personal communication.

Malik S, Kenny M and Ahmad S (1993) A convenient method to measure di-(2-ethylhexyl) phthalate from the serum of hemodialysis patients. Toxicol. Environ. Chem. **37**, 133-137.

Malish R (1981) Sedimente als Modelle für die Berteilung der Umweltkontamination durch chlororganische Pestizide, polychlorierte Biphenyle und Phthalate unter besonderer Berücksichtigung des zeiylichen Verlaufs. Dissertation. Münster.

Mangham BA, Foster JR and Lake BG (1981) Comparison of the hepatic and testicular effects of orally administered di(2-ethylhexyl) phthalate and dialkyl 79 phthalate in rat. Toxicol. Appl. Pharmacol. **61**, 205-214.

Mann AH, Price SC, Mitchell FE, Grasso P, Hinton RH and Bridges JW (1985) Comparison of the shor-term effects of di-(2-ethylhexyl) phthalate, di(n-hexyl) phthalate and di(n-octyl) phthalate in rats. Toxicol. Appl. Pharmacol. **77**, 116-132.

Marcel YL (1973) Detemination of di(2-ethylhexyl)phthalate levels in human blood plasma and cryoprecipitates. Environ Health. Perspect 4(3), 119.

Marin ML et al. (1998) Analysis of Potentially Toxic Phthalate Plasticizers Unsed in Toy Manufacturing. Environ. Contam. Toxicol 60:68-73.

Marsman DS and Popp JA (1994) Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator Wy-14,643. Carcinogenesis 15, 111-117.

Marsman DS, Cattley RS, Conway JG and Popp JA (1988) Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferator di(2-ethylhexyl) phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643) in rats. Cancer Research **48**, 6739-6744.

Marsman DS, Goldsworthy TL and Popp JA (1992) Contrasting hepatocytic peroxisome proliferation, lipofuscin accumulation, and cell turnover for the hepato-carcinogens Wy-14,643 and clofibric acid. Carcinogenesis 15, 1011-1017.

Marwedel G; Chem Ztg /Chem App 90 (1966) 723

Mathur SP (1974) Respirometric evidence of the utilization of Di-octyl and Di-2-ethylhexyl Phthalate Plasticizers. J. Environ. Quality, Vol. 3, 207-209.

Matsushima T, Muramatsu M and Haresaku M (1985) Mutation tests on *Salmonella typhimurium* by the preincubation method. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 181-186.

Matthews EJ, DelBalzo T and Rundell JO (1985) Assays for morphological transformation and mutation to ouabain resistance of Balb/c-3T3 cells in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 639-650.

Mattson M (1999) Personal communication. From "The Assosiation of Swedish Automobil Manufactures and Whole salers". 1999-01-27.

Mayer et al. (1977) Recent advances in fish toxicology. A symposium held in corvallis, Oregon on January 13-14, 1977. Corvallis environmental research lab, Oregon. NTIS, PB-273 500.

Mayer FL (1976) J. Fish. Res. Board Can.33, 2610-2613, cited in: Veith,G.D. et al., J. Fish. Res. Board Can.36, 1040-1048, (1979).

Mayer FL and Sanders HO (1973) Toxicology of pthalic acid esters in aquatic organisms. Environ. Health Perspect. **3**, 153-157.

Mayer FL Jr, Stalling DL and Johnson JL (1972) Phthalate esters as environmental contaminants. Nature (London), 238, 411-413.

Mayer FL, Mehrle PM and Schoettger RA (1977) Collagen in fish exposed to organic chemicals. Recent advances in fish toxicology. A symposium held in Corvallis, Oregon on January 13-14, 1977. Corvallis environmental research lab., Oregon . NTIS, PB-273 500.

McLachlan MS (1996) Bioaccumulation of Hydrophobic Chemicals in Agricultural Food Chains. Env. Sc. Tech. Vol. 30 no.1, 252-259.

Mehrle PM and Mayer FL (1976) Di-2thylhexyl phthalate: Residue dynamics and biological effects in Rainbow tout and Fathead minnows. Trace Substances and Environmental Health **10**, 519-524.

Mehta RD and von Borstel RC (1985) Tests for genetic activity in the yeast *Saccharomyces cerevisiae* using strans D7-144, XV185-14C and RM52. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 271-284.

Meki J-C (1999) PLANIA AB Luleå (Swedish plastrisol processor). Personal communication.

Melnick RL, Morrissey RE and Tomaszewski KE (1987) Studies by the National Toxicology Program on di-(2-ethylhexyl) phthalate. Toxicol. Ind. Health **3**, 99-118.

Menzel B (1997) Dampfdruck von Di-Ethylhexylphtalate (DOP). Hüls report 15.01.1997.

Menzel B (1995) Determination of the acute effects of VESTINOL AH on fish. Short-term test as specified by 92/69 C1 EEC. Hüls AG. Final Report No. FK 1319,

Merkle J, Klimisch HJ and Jäckh R (1988) Developmental toxicity in rats after inhalation exposure of di(2-ethylhexyl) phthalate (DEHP). Toxicol. Lett. **42**, 215-223 (1988).

Metcalf RL, Booth GM, Schuth CK, Hansen DJ and Lu P-Y (1973) Uptake and fate of Di-2-ethylhexyl phtalate in aquatic organisms and in a model ecosystem. Univ. of Illinois and Illinois Natural History Survey. Environ. Health Perspect. X, 27-34.

Metcalfe CD, Metcalfe TL, Kiparassis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE and Potter T (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluent as determined by in vivo assays with japanese medaka (*Oryzias latipes*). Environmental toxicology and chemistry vol. 20, No 2, pp 297-308.

Meylan W, Howard PH and Boethling RS (1992) "Molecular Topology/Fragment Contribution Method for Predicting Soil Sorption Coefficients", Environ. Sci. Technol. 26: 1560-7.

Mieure JP and Dietrich MW (1973) Determination of trace organics in air and water. J. Chromatogr. Sci. 11:559-570.

Mikalsen S-O and Sanner T (1993) Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. Carcinogenesis **14**, 251-257.

Mikalsen S-O, Holen I and Sanner T (1990a) Morphological transformation and catalase activity of Syrian hamster embryo cells treated with hepatic peroxisome proliferators, TPA and nickel sulphate. Cell Biol. Toxicol. 6, 6-13.

Mikalsen S-O, Kaalhus O, Reith A and Sanner T (1990b) Role of catalase and oxidative stress in hepatic peroxisome proliferator-induced morphological transformation of Syrian hamster embryo cells. Int. J. Cancer 46, 950-957.

Mikalsen S-O, Ruyter B and Sanner T (1990c) Effects of hepatic peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on catalase and other enzyme activities of embryonic cells in vitro. Biochem. Pharmacol 39, 527-535.

Mikkelsen J (1995) Fate model for Organic Chemicals in Sewage Treatment Plants. Environmental project No. 308.

Mikkelsen J, Nyholm N, Neergaard-Jacobsen B and Chen-Fredenslund F (1996) Evaluation and modification of the SIMPLETREAT chemical fate model for activated sludge sewage tratment plants. Wat. Sci. Tech. vol.33 p. 279-287.

Miljöstyrelsen (1996) Masseströmsanalyse for phthalater. Miljöprojekt Nr. 320, 1996. fra Miljöstyrelsen. Denmark (in Danish).

Miljöstyrelsen (1997) Baeredygtighed af nuvaerende miljöbelastning med persistente kemiska stoffer. Arbejdsrapport fra Miljöstyrelsen. No. 52. Denmark.

Milkov LE, Aldyreva MV, Popova TB, Lopukhova KA, Makarenko YL, Malyar LM and Shakhova TK (1973) Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins. Environ. Health Perspect. **3**, 175-178.

Miller RT and Cattley RC (1996) Characterization of regressing peroxisome proliferator induced hepatocellular adenomas. Proc. AARC 37, 160.

Ministeriet for Fødevarer, Landbrug og Fiskeri Veterinær- og Fødevaredirektoratet (1998). Analyse af plastblødgørere i modermælkserstatninger, børnemad og dagskost (Plasticisers in Infant Fomulae, Baby Food and Total Diet Samples) Intern rapport IFE 1998.4

Miripol JE and Stern IJ (1977) Decreased accumulation of phthalate plasticizer during storage of blood as packed cells . Transfusion 17, 17-72.

Mitchell AM, Lhuguenot J-C, Bridges JW and Elcombe CR (1985b) Identification of the proximate peroxisome proliferator(s) derived from di(2-ethylhexyl) phthalate. Toxicol. Appl. Pharmacol. **80**, 23-32.

Mitchell FE, Price SC, Hinton RH, Grasso P and Bridges JW (1985a) Time and dose-response study of the effects on rats of the plasticizer di-(2-ethylhexyl) phthalate. Toxicol. Appl. Pharmacol. **81**, 371-392.

MITI, Ministry of International Trade and Industry Japan (1992). Data of Existing Chemicals based on the CSCL Japan, pp 3-90.

Mitsubishi Chemical Co. (1995) Thirteen week repetitive oral administration test of di(2-ethylhexyl)phthalate using marmosets. Test Report (Test Number 4L209), K.K. Mitsubishi Kagaku Anzen Kakaku Kenkyujo, Kashima Laboratory. Plastizied Industries Association. 90pp.

Miura R et al. (1993) Process for producing sinters and binders for use in that process. United States Patent: 5,266,264. US Patent and Trademark Office. November 30, 1993.

Miyazawa S, Furuta S, Osumi T and Hashimoto T (1980) Turnover of enzymes of peroxisomal β-oxidation in rat liver. Biochim. Biophys. Acta **630**, 367-374.

Monsanto (1988) Effect of repeated oral dosing on the disposition and metabolism of DEHP in monkeys. TSCATS: OTS 0516749, Doc. I. D.: 86-890000212, 08-31-88, Monsanto Co.

Moody DE and Reddy JK (1978) Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds. Toxicol. Appl. Pharmacol. **45**, 497-504.

Moore at el. (2001) Environmental Health Perspectives, 109, 229-237

Moore MR (1996) Oncogenicity study in rats with Di (2-ethylhexyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Corning Hazleton Incorporated (CHV), 9200 Leesburg Pike, Vienna, Virginia 22182-1699. Laboratory Study Identification: CHV 663-134; Sponsor: Eastman Chemical Company, First America Center, P.O. Box 1994 Kingsport, Tennessee 37662-5394

Moore MR (1997) Oncogenicity study in mice with Di (2-ethylhexyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses. Corning Hazleton Incorporated (CHV), 9200 Leesburg Pike, Vienna,

Virginia 22182-1699. Laboratory Study Identification: CHV 663-135; Sponsor: Eastman Chemical Company, First America Center, P.O. Box 1994 Kingsport, Tennessee 37662-5394

Morita MH, Nakamura H and Mimura S (1974) Phthalic acid esters in water. Water res. 8, 781.

Morrissey RE, Lamb IV JC, Schwetz BA, Teague JL and Morris RW (1988) Association of sperm, vaginal cytology, and reproductive organ weight data with results of continuous breeding reproduction studies in Swiss (CD-1) mice. Fund. Appl. Toxicol. **11**, 359-371.

Mortensen GK, Main KM, Andersson A-M, Leffers H and Skakkebaek NE Anal Bioanal Chem (2005), 382(4), 1084-1092, Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS)

Moser VC, Cheek BM and MacPhail RC (1995) A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity. J. Toxicol. Environ. Health **45**, 173-210.

Moss EJ, Cook MW, Thomas LV and Gray TJB (1988) The effect of mono-(2-ethyl) phthalate and other phthalate esters on lactate production by Sertoli cells *in vitro*. Toxicol. Lett. **40**, 77-84.

MST (1995) Male reproductive health and environmental chemicals with estrogenic effects. Danish Environmental Protection Agency, Miljøprojekt nr. 290.

Mutz RC and Jones JR (1977) The effects of phthalate esters on geochemical cycles in freshwater hydrosoil. Transactions of the Missouri Academy of Science. Vol 10 and 11, p. 296.

Myhr B, Bowers L and Caspary WJ (1985) Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 555-568.

Mylchreest E and Foster PMD (1998) Antiandrogenic effects of di(*n*-butyl) phthalate on male reproductive development: a nonreceptor-mediated mechanism. CIIT Activities **18** (9), 1-8.

Mylchreest E, Sar M, Cattley RC and Foster PMD (1999) Disruption of Androgen-Regulated Male Reproductive development by Di(n-Butyl) Phthalate during Late Gestation in Rats Is Different from Flutamide. Toxicology and Applied Pharmacology 156, 81-95.

Nagaoka et al. (1996) Plant Growth Regulation 20 p.295-302.

Nakamura Y, Yagi Y, Tomita I and Tsuchikawa K (1979) Teratogenicity of di(2-ethylhexyl) phthalate in mice. Toxicol. Lett. **4**, 113-117.

Narotsky MG, Weller EA, Chinchilli VM and Kavlock RJ (1995) Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 x 5 x 5 design. Fundam. Appl. Toxicol. **27**, 203-216.

Nässberger L, Arbin A and Östelius J (1987) Exposure of patients to phthalates from polyvinyl chloride tubes and bags during dialysis, Nephron 45, 286-290.

Natow M (1981) Über das Auswandern von Weichmachern aus Polyvinylchloriderzeugnissen, Plaste Kautschuk 28, 459-460.

Naylor LM and Loehr RC (1982) Biocycle July/August, 8.

Neff et al. (1984) Neff, J.M. and Fresenius, Z. Anal. Chem. 319, 132.

Nelson BK et al. (1989) Developmental toxicicology evaluation of 1-Pentanol,1-Hexanol, and 2-Ethylhexanol administered by inhalation to rats. J AM Coll Toxicol 8; 405-410.

Neuhauser EF, Loehr RC, Malecki MR, Milligan DL and Durkin PR (1985) The toxicity of selected organic chemicals to the earthworm Eisenia fertida. J. Environ. Qual.; Vol. 14, 383-388.

Ng KME, Chu I, Bronaugh RL, Franklin CA and Somers DA (1992) Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di-(2-ethylhexyl) phthalate: Comparison of *in vitro* and *in vivo* results in the hairless guinea pig. Toxicol. Appl. Pharmacol. **115**, 216-223.

Nielsen J, Åkesson B and Skerfving S (1985) Phthalate ester Exposure- air Levels and Health of Workers Precessing Polyvinylchloride. Am. ind. Hyg. Assoc. J. 46(11), 643-647.

Nilsson R, Beije B, Préat V, Erixon K and Ramel C (1991) On the mechanism of the hepatocarcinogenicity of peroxisome proliferators. Chem.-Biol. Interaction, 78, 235-250.

Norman A, Börjeson H, David F, Tienpont B and Norrgren L (2007) Studies of uptake, elimination and late effects in Atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology (AECT).

Norrgren L, Blom A, Andersson PL, Börjesson H, Larsson DGJ and Olsson P-E (1999) Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (*Salmo salar*). Aquatic Ecosystem Health and Management, Vol 2/3. pp.311-317.

NTIS (National Technical Information Service) (1984) Teratologic evaluation of diethylhexyl phthalate (CAS No. 117-81-7) in CD-1 mice. PB85-105674, 06-20-84.

NTIS (National Technical Information Service) (1988) Reproduction and fertility evaluation of diethylhexyl phthalate (CAS No 117-81-7) in CD-1 mice exposed during gestation. PB88-204300, 04-15-88.

NTP (National Toxicology Program) (1982a) Carcinogenesis bioassay of di(2-ethylhexyl) phthalate in F344 rats and B6C3F1 mice (feed study). NTP Technical Report No. 217, 01-82.

NTP (National Toxicology Program) (1982b) Carcinogenesis bioassay of Butyl benzyl phthalate in F344 rats and B6C3F1 mice (feed study). NTP Technical Report No. 213, 01-98.

NTP (National Toxicology Program) (1985) Toxicology and Carcinogenesis studies of Diallylphthalate in F344 rats (Gavage studies). NTP Technical Report No. 284, 01-154.

NTP (National Toxicology Program) (1991) Final Report on the developmental toxicity of mono(2-ethylhexyl) phthalate in CD-1 Swiss mice.

NTP (National Toxicology Program) (1997) Toxicology and Carcinogenesis studies of Butyl benzyl phthalate in F344 rats (feed study). NTP Technical Report No. 458, 01-193.

Nuodex (1980) Salmonella/mammalian microsome plate incorporation mutagenesis assay. TSCATS: 206260, Doc. I. D.: 878210238, 11-21-80, Nuodex Inc.

Nuodex (1981a) Acute oral toxicity study in rats of TCI compounds: R-1268, R-1272, R-1286 and R-1287. TSCATS: 206260, Doc. I. D.: 878211655, 06-02-81, Nuodex Inc.

Nuodex (1981b) Dominant lethal study of three compounds: R1213, R-1214 and R-1217. TSCATS: 206260, Doc. I. D.: 878210249, 05-01-81, Nuodex Inc.

Nuodex (1981c) Repeated dose 28-day toxicity study with rodents. TSCATS: 206260, Doc. I. D.: 878211342, 06-06-81, Nuodex Inc. Unpublished study.

Nuodex (1981d) Evaluation of test article R-1217 for mutagenic potential employing the L5178 TK+/- mutagenesis assay. TSCATS: 206260, Doc. I. D.: 878210241, 01-21-81, Nuodex Inc.

Nuodex (1981e) Evaluation of R-1217 in the primary rat hepatocyte unscheduled DNA synthesis assay. TSCATS: 206260, Doc. I. D.: 878210239, 02-01-81, Nuodex Inc.

Nuodex (1981f) Activity of T1642 in the in vitro mammalian cell transformation assay in the presence of exogenous metabolic activation. TSCATS: 206260, Doc. I. D.: 878210243, 04-30-81, Nuodex Inc.

Nuodex (1981g) Evaluation of R-1269 in the in vitro transformation of Balb/c-3T3 cells assay. TSCATS: 206260, Doc. I. D.: 878210245, 07-01-81, Nuodex Inc.

Nuodex (1981h) Evaluation of R-1217 in the in vitro transformation of Balb/c-3T3 cells assay TSCATS: 206260, Doc. I. D.: 878210244, 06-01-81, Nuodex Inc.

Nuodex (1981i) Activity of T1648 in the iv vivo cytogenetics assay in rodents. TSCATS: 206260, Doc. I. D.: 878210240, 03-27-81, Nuodex Inc.

O'Connor OA, Rivera MD and Young LY (1989) Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environmental Toxicology and Chemistry, Vol 8. pp. 569-576.

OAEI (1996). O´conner Associates Environmental Inc. Report No. 10-4046. March 1996.

Obe G, Hille A, Jonas R, Schmidt S and Thenhaus U (1985) Tests for the induction of sister-chromatid exchanges in human peripheral lymphocytes in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 439-442.

Oberly TJ, Bewsey BJ and Probst GS (1985) Tests for the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 569-582.

Occupational Exposure to Phthalate Esters with specific reference to Di-ethyl Hesile Phthalate (DEHP), Di-Butyl Phthalate (DBP), Di-isononyl Phthalate (DINP), and Di-Isodecyl Phthalate (DIDP), Includes 3 append.

OECD (1981) OECD-Laboratory Intercomparision Testing Programme, Part I 1979/80, Part II 1980/81, Initiated by the OECD Chemicals Testing Programme, Expert Group A "Physico-Chemical Properties".

Oesterle D and Deml E (1988) Promoting activity of di-(2-ethylhexyl) phthalate in rat liver foci bioassay. J. Cancer Res. Clin. Oncol. **114**, 133-136.

Øie L, Hersoug L-G and Ogaard Madsen J (1997) Residential exposure to plasticisers and its possible role in the pathogenesis of asthma. Environmental Health Perspectives. 105:972-978.

Oishi S (1985) Reversibility of testicular atrophy induced by di(2-ethylhexyl) phthalate in rats. Environ. Research **36**, 160-169.

Oishi S (1986) Testicular atrophy induced by di(2-ethylhexyl) phthalate: Changes in histology, cell specific enzyme activities and zinc concentrations in rat testis. Arch. Toxicol. **59**, 290-295.

Oishi S (1990) Effects of phthalic acid esters on testicular mitochondrial functions in the rat. Arch. Toxicol. **64**, 143-147.

Oishi S (1993) Strain differences in susceptibility to di-2-ethylhexyl phthalate-induced testicular atrophy in mice. Toxicol. Lett. **66**, 47-52.

Oishi S (1994) Prevention of di(2-ethylhexyl) phthalate-induced testicular atrophy in rats by co-administration of the vitamin B_{12} derivative adenosylcobalamin. Arch. Environ. Contam. Toxicol. **26**, 497-503.

Oishi S and Hiraga K (1982) Distribution and elimination of di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) after a single oral administration of DEHP in rats. Arch. Toxicol. **51**, 149-155.

Oishi S and Hiraga K (1980) Effect of phthalic acid esters on mouse testes. Toxicol. Lett. **5**, 413-416.

Oishi S and Hiraga K (1983) Testicular atrophy induced by di-2-ethylhexyl phthalate: Effect of zinc supplement. Toxicol. Appl. Pharmacol. **70**, 43-48.

Öman and Hynning (1993) in Lundberg, G. And Nilsson C. (1994).

Ono K, Tatsukawa R and Wakimoto T (1975) Migration of plasticizer from hemodialysis blood tubing, J Am. Med. Assoc. 234, 948-949.

Ormstad H, Gaarder PI and Johansen BV (1997) Quantification and characterisation of suspended particulate matter in indoor air. The Science of the Total Environment. 193. 185-196.

Page BD and Lacroix GM (1992) Studies ointo the transfer and migration of phthalate esters from aluminium foil-paper laminates to butter and margarine. Food Additives and Contaminants 9, 197-212.

Page BD and Lacroix GM (1995) The Occurence of Phthalate Ester and di-2-ethylhexyl Adipate Plasticizers in Canadian Packaging and Food Sampled in 1985-1989: a Survey. Food Additives and Contaminants, 12:1. 129-151.

Palmer CNA, Hsu M-H, Griffin KJ, Raucy JL and Johnson EF (1998) Peroxisome Proliferator Activated Receptor-α Expression in Human Liver. Mol. Pharmacol. 53: 14-22.

Park CW, Imamura H and Yoshida T (1990) Uptake, excretion and metabolism of 14C-labelled di-2-ethylhexyl phtalate by Mullet, *Mugil cephalus*. Korea ocean Research and Development Institute. Bull. Korean Fish. Soc. 22(6), 424-428

Parkman H and Remberger M (1996) Phtalate esters in water and sediments in major cities and remote lakes in Sweden

Parkman H and Remberger M (1995) Phthalates in Swedish Sediments, Swedish Environmental Research Institute, IVL Report B 1167.

Parks et al. (2000) Toxicological Sciences, 59, 339-349

Parmar D, Srivastava SP and Seth PK (1986) Effect of di(ethylhexyl) phthalate (DEHP) on spermatogenesis in adult rats. Toxicol. **42**, 47-55.

Parmar D, Srivastava SP, Singh GB and Seth PK (1987) Effect of testosterone on the testicular atrophy caused by di(2-ethylhexyl) phthalate (DEHP). Toxicol. Lett. **36**, 297-308.

Parmar D, Srivastava SP, Srivastava SP and Seth PK (1985) Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to di-(2-ethylhexyl) phthalate through mother's milk. Drug Metabol. Dispos. **13,** 368-370.

Parmar D, Srivastava SP, Srivastava SP and Seth PK (1995) Testicular toxicity of di(2-ethylhexyl) phthalate in developing rats. Vet. Human. Toxicol. **37**, 310-313.

Parry EM (1985) Tests for effects on mitosis and the mitotic spindle in Chinese hamster primary liver cells (CH1-L) in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 479-485.

Parry JM and Eckardt F (1985) The detection of mitotic gene conversion, point mutation and mitotic segregation using the yeast *Saccharomyces cerevisiae* strain D7. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 261-269.

Parry JM, Danford N and Parry EM (1984) *In vitro* techniques for the detection of chemicals capable of inducing mitotic chromosome aneuploidy. ATLA **11**, 117-128.

Passino DRM and Smith SB (1987) Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. Environmental Toxicology and Chemistry, Vol. 6, pp 901-907.

Pastuska G, Bahrnheim D und Just U (1984) Zur Langzeitverhalten von Bahnen aus PVC-Weich, Teil I Kautschuk+Gummi-kunststoffe 37, p 703-705.

Pastuska G, Kerner-Gang W und Just U (1988) Langzeitverhalten von PVC-P-Dachbahnen, Kautschuk+Gummi-kunststoffe 41, 451-454.

Pastuska G and Just U (1990) Weichmacherverlust aus langjährig freibewitterten PVC-P-Dachbahnen. Kautschuk+Gummi-kunststoffe 43, p.1093-1094.

Peakall D (1975) Phthalate esters: occurence and biological effects. Residue reviews 54, 1.

Pearson SD and Trissel LA (1993) Leaching of diethylhexyl phthalate from polyvinyl chloride containers by selected drugs and formulation components. Am. J. Hosp. Pharm. 50, 1405-1409.

Peck CC and Albro PW (1982) Toxic potential of the plasticizer di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. Environ. Health Perspect. **45**, 11-17.

Peck CC, Albre PW, Hass JR Odom DG, Barrett BB and Bailey FJ (1978) Metabolism and excretion of the plasticiser di-2-erthylhexylphthalate. Clin. Res. 26 (2), 101A

Peck CC et al. (1979) Di-(2-ethylhexyl)phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion 19, 137-146.

Pedersen F (1996) Review of Environmental Fate and Effects of di(2-ethylhexyl)phtalate. Arbejsraport fra Miljöstyrelsen No 54 1996 + Comments from the ECPI (European Council for Plasticisers and Intermediates)

Pelling D, Phillips JC and Cunninghame ME (1998) Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin *in vitro*. Toxicology *in vitro* 12, 47-55

Perez KT, Davey EW, Lackie NF, Morrison GE, Murphy PG, Soper AE and Windslow DL (1983) Environmental assessment of a phthalate ester, Di(2-ethylhexyl) phthalate (DEHP), derived from a marine microcosm. Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802, W. E. Bishop, R.D. Cardwell, and B. B. Heidolph (Eds.), American Society for Testing and Materials, Philadelphia, pp 180-191. 1983 (ASTM Special technical publ., 0066-0558;802)

Persson PE, Penttinen H and Nuorteva P (1978) DEHP in the vicinity of an industrial area in Finland. Environ. Pollut., 16, 163-166.

Perwak J, Goyer M, Schimke G, Eschenroeder A, Fiksel J, Scow K and Wallace D Slimak M Project Manager. (1981). EPA-440/4-81-020.

Peters JM, Cattley RC and Conzalez FJ (1997) Role of PPARα in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. Carcinogenesis, 18, no.11, 2029-2033.

Peters JM, Wiley LM, Zidenberg-Cherr S and Keen CL (1995) Periconceptional zinc deficiency affects uterine 3H-estradiol binding in mice. Terato. Carcino. Mutagen., 15: 25-31.

Petersen JH (1991) Survey of di-(2-ethylhexyl)phthalate plasticizer contamination of retail Danish milks. Food Additives and Contaminants 8(6), 701-706.

Petersen JH (2000) Plasticizers in total diet samples, baby food and infant formulae Food Addit Contam; Vol 17, Issue 2, pp 133-41.

Pfab W (1967) Migration of phtalate plasticizers from lacquered foils on fatty foods (transl). Deut. Lebensm.-Runsch. 63 (3), 72.

Pfannhauser W, Leitner E, Mayer I And Schaffer A (1997) Phthalate in Süsswasser ishen Österreichischer herkunft. Schriftenreihe des Bundesministerium für Umwelt, Jugend und Familie band 18/1997. ISBN 3-901 305-69-6.

Phillips BJ, James TEB and Gangolli SD (1982) Genotoxicity studies of di(2-ethylhexyl) phthalate and its metabolites in CHO cells. Mutation Research **102**, 297-304.

Phtalater afgivet ved vask af PVC-holdige gulve og textiler, Danmarks Miljøundersøgelser, 8 september 1995.

Phthalic acid, di(2-ethylhexyl)ester (DEHP): Study of Embryo-Foetal Toxicity in the CD-1 Mouse by Oral Gavage Administation. Huntington Life Sciences Ltd 1996, sposored by Elf Atochem SA.

Piechocki JT and Purdy WC (1973) Determination of di-(2-ethylhexyl)-phthalate (DEHP) in human plasma. Clin. Chinr. Acta. 48:385-391.

Pik J, Braier J, Kalman Z and Jozef M (1979) Determination of bis(2-ethylhexyl)phthalate (DEHP) in blood preparations stored in poly(vinyl chloride) bags (Rum.), Probl. Gematol. Per. Krovi., 24:49-51, 1979. Chem. Abstr., 91:27213n)

Plate D (1997(?)). Schadstofffreisetzung aus PVC-weich-Folien unter Deponiemilieu-Bedungungen. No. 10 in "AJ"(?), p.10-15. Copy from C. Schwarz, BASF AG.

Plonait SL, Nau H, Maier RF, Wittfoht W and Obladen M (1993) Transfusion 33, 598-605.

Pollack GM, Buchanan JF, Slaughter RL, Kohli RK and Shen DD (1985b) Circulating concentrations of di-(2-ethylhexyl) phthalate and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. Toxicol. Appl. Pharmacol. **79**, 257-267.

Pollack GM, Li RCK, Ermer JC and Shen DD (1985a) Effects of route of administration and repetitive dosing on the disposition kinetics of di(2-ethylhexyl) phthalate and its mono-de-esterified metabolite in rats. Toxicol. Appl. Pharmacol. **79**, 246-256.

Poon R, Lecavalier P, Mueller R, Valli VE, Procter BB and Chu I (1997) Subchronic oral toxicity of di-*n*-octyl phthalate and di(2-ethylhexyl) phthalate in the rat. Food Chem. Toxicol. **35**, 225-239.

Popp JA, Garvey LH, Hamm Jr TE and Swenber JA (1985) Lack of hepatic promotional activity by the peroxisomal proliferating hepatocarcinogen di-(2-ethylhexyl) phthalate. Carcinogenesis **6**, 141-144.

Préat V, Lans M, de Gerlache J, Taper H and Robertfroid M (1986a) Comparation of the biological effects of phenobarbital and nafenopin on rat hepatocarcinogenesis, Japanese J. Cancer Res. 77, 629-638.

Préat V, Lans M, de Gerlache J, Taper H and Robertfroid M (1986b) Comparative analysis of the effect of phenobarbital, dichlorodiphenyltrichloroethane,butylated hydroxytoluene and nafenopin on rat hepatocarcinogenesis, Carcinogenesis, 7, 1025-1028.

Price CJ et al. (1991a) Teratology 43, 457 (Abstract No. P132).

Price CJ, Marr MC, Myers CB, Morrissey RE, Heindel JJ and Schwetz BA (1991b) Developmental toxicity evalution of mono (2-ethylhexyl)phthalate (CAS no. 4376-20-9) in CD-1-Swiss mice: volume 1 and 2, final study report and appendix. NTIS Technical Report (NTIS/PB91-185926) 1991 Jan: 162 pp.

Priston RAJ and Dean BJ (1985) Tests for the induction of chromosome aberrations, polyploidy and sister-chromatid exchanges in rat liver (RL_4) cells. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 387-395.

Probst GS and Hill LE (1985) Tests for the induction of DNA-repair synthesis in primary cultures of adult rat hepatocytes. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 381-386 (1985).

Pugh G et al. (2000) Effects of Di-isononyl Phthalate, Di-2-ethylhexyl Phtalate, and Clofibrate in Cynomolgus Monkeys. Toxicological Sciences, 56, 181-188.

Putman DL, Moore WA, Schechtman LM and Hodgson JR (1983) Cytogenetic evaluation of di(2-ethylhexyl) phthalate and its major metabolites in Fischer 344 rats. Environ. Mutagen. **5**, 227-231.

Quackenbos HM Jr (1954) Plasticizers in vinyl Chloride resins. Migration of plasticizer. Industrial and Engineering Chemistry 46:1, 1335-1344.

Rüdel H and Waymann B (1992) Volatility of pesticides in a wind tunnel. Brighton Crop Protection Conference -– Pest and Deseases -– 1992. p. 841-846.

Rüdel H, Schmidt W and Klein W (1993) Degradation of pesticides in soil: comparison of laboratory experiments in a biometer system and outdoor lysimeter experiments. The Science of the Total Environment, 132, p. 181-200.

Rao et al. (1985) in "TSD (1991)".

Rao MS, Usuda N, Subbarao V and Reddy JK (1987) Absence of γ-glutamyl transpeptidase activity in neoplastic lesions induced in the liver of male F-344 rats by di(2-ethylhexyl) phthalate, a peroxisome proliferator. Carcinogenesis **8**, 1347-1350.

Rao MS, Yeldandi AV and Subbarao V (1990) Quantitative analysis of hepatocellular lesions induced by di-(2-ethylhexyl) phthalate in F-344 rats. J. Toxicol. Environ. Health **30**, 85-89.

Rastogi SC, Vikelsöe J, Jensen G, Johansen E and Carsen L (1997) Migration of phthalates from teethers. research Notes from NERI No. 54. Ministry of Environment and Energy. National Environmental Research Institute.

Rastogi SC, Worsöe IM, Köppen B, Hansen AB and Avnskjold J (1998) Inhold af organiske oplösningsmidler og phthalater i legetöj. Analytsk-kemisk kontrol af kemiske stoffer og produkter. Miljö- og Energiministeriet, Danmarks Miljöundersøgelser.

Rathleff-Nielsen B (1999) Rapport rörande Plastisolbelagd Stålplåt *(about repainting steel coated with plastisol)*. BRN-Service. 1998.06.25 *(only in Swedish)*

Reddy JK and Lalwani ND (1983) Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. Crit. Revs. Toxicol. 12, 1-58.

Reddy TV, Chang LW, DeAngelo AM, Pereira MA and Daniel FB (1992) Effect of nongenotoxic environmental contaminants on cholesterol and DNA synthesis in cultured primary rat hepatocytes. Environ. Sci. **1**, 179-189.

Renner I et al. (1990) VDI-Bericht 837, 705-727, zitiert nach Rippen: Handbuch Umweltchemikalien, 3.Erg.Lfg.1/92

Rexroat MA and Probst GS (1985) Mutation tests with Salmonella using the plate-incorporation assay. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 201-212.

Rhodes JE, Adams WJ, Biddinger GR, Robillard KA and Gorsuch JW (1995) Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry, Vol. 14, No 11, pp 1967-1976.

Rhodes C, Elcombe CR, Batten PL, Bratt H, Jackson SJ, Pratt IS and Orton TC (1983) The disposition of ^{14}C-di-2-ethylhexylphthalate (DEHP) in the marmoset. Dev. Sci. Pract. Toxicol. **11**, 579-581.

Rhodes C, Orton TC, Pratt IS, Batten PL, Bratt H, Fackson SJ and Elcombe CR (1986) Comparative pharmacokinetics and subacute toxicity of di-(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. Environ. Health Perspect. **65**, 299-308.

Rhodes JE and McAllister WA (1990) Preliminary investigation of the toxicity of Di-(2-ethylhexyl)phthalate (DEHP) to the Rainbow trout (Onchorhynchus mykiss) under flow-through conditions. ABC inc. ABC report #36568. Sponsored by BASF AG. BASF project 52FO7822/875236.

Rhodes JE, Adams WJ, Biddinger GR, Robillard KA and Gorsuch JW (1995) Chronc Toxicity of 14 Phthalate esters to Daphnia magna and Rainbow Trout (Oncorhynchus mykiss), Environmental Toxicology and Chemistry, Vol. 14, No. 11, pp. 1967-1976.

RIC (2000a) Overview of phthalate measurements in air, Research Institute of Chromatography, Kortrijk, Belgium, 3pp (cited in ECPI 2000).

RIC (2000b) Determination of phthalates in fish samples. Letter to CEFIC, 29-8-2000. O.Ref: ECPI-2000-07

RIC (2000c) Determination of phthalates in fish samples. Letter from F. David, Research Institute of Chromatography, Kortrijk, Belgium to CEFIC. RIC ref: ECPI-2000-11.

RIC (2001a) RIVM monitoring program for milk, vegetation, cattle feed, fish and air (subproject E2). Part F: Sediment samples in conjunction with fish and invertebrate samples. Letter from F. David, Research Institute of Chromatography, Kortrijk, Belgium to CEFIC. RIC ref: ECPI-2001-05.

RIC (2001b) Part 3b: Air sampling and analysis – winter 2001. Letter from F. David, Research Institute of Chromatography, Kortrijk, Belgium to CEFIC. RIC ref: ECPI-2001-01.

RIC (2001c) RIVM monitoring program for milk,vegetation,cattle feed, fish and air (subproject E2). Part 2E: Invertebrate samples. Letter from F. David, Research Institute of Chromatography, Kortrijk, Belgium to CEFIC. RIC ref: ECPI-2001-04.

RIC (2001d) RIVM monitoring program for milk,vegetation,cattle feed, fish and air (subproject E2). Part 2 D: Fish samples – additional samples. Letter from F. David, Research Institute of Chromatography, Kortrijk, Belgium to CEFIC. RIC ref: ECPI-2001-03.

Richburg JH and Boekelheide K (1996) Mono-(2-ethylhexyl) phthalate rapidly alters both Sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. Toxicol. Appl. Pharmacol. **137**, 42-50.

Riddick JA, Bunger WB and Sakano TK (1986) Organic Solvents: Physical Properties and Methods of Purification-Techniques of Chemisty, 4th edition, New York, Wiley-Interscience 2a, 443.

Rippen (1992) Handbuch Umweltchemikalien, 13.Erg.Lfg.1/92.

Rippen (2005) Handbuch Umweltchemikalien, 13.Erg.Lfg.1/92. Latest update no 72 (2005)

Ritchlin J and Holmes M (1999) Monitoring Solid Pulp Mill Wastes. Information in a Internet home site, Oct 1999. Adress/phones: 1672 East Tenth Ave., Vancouver V5N 1X5 ph: (604) 879-2992, fax: (604) 879-2272.

Ritsema R, Cofino WP, Frintrop PCM and Brinkman UATh (1989) Trace-levels of phtalate esters in surface water and suspended particulate matter by means of capillary gas chromatography with electron-capture and mass-selective detection. Rijkswaterstaat and Free University of Amsterdam, the Netherlands. Chemosphere **18** (11/12), 2161-2175.

Ritter EJ, Scott WJ Jr, Randall JL and Ritter JM (1987) Teratogenicity of di(2-ethylhexyl) phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, and valproic acid, and potentiation by caffeine. Teratology **35**, 41-46.

RIVM (1992) Toxicological investigation of di(2-ethylhexyl) phthalate in rats. The determination of a no-observed-effect-level. Report nr 618902 007 from the National Institute of Public Health and Environmental Protection to the Dutch Chief Inspectorate of Health Protection.

RIVM (Rijksintituut voor volksgezondheid en milieu) (1997). Risk assessment report for dibutylphthalate. Draft of 17 November 1997.

RIVM/CSR -– ECB (1998) BLACKLIST EUSES 1.00. list of bugs in the program. 15 june 1998.

Rock G, Labow RS and Tocchi M (1986) Distribution of di(2-ethylhexyl) phthalate and products in blood and blood components, Environ. Health Perspect. **65**, 309-316.

Rock G, Secours NE, Franklin CA, Chu I and Villeneuve DC (1978) The accumulation of mono-2-ethylhexylphthalate (MEHP) during storage of whole blood and plasma Transfusion. **18**, 553-558.

Rock G, Tocchi M, Ganz PR and Tackaberry ES (1984) Incorporation of plasticiser into red cells during atorage. Transfusion **24** (6), 493-498.

Rodent Leydig Cell Tumorigenesis: A Review of the Physiology, Pathology, Mechanisms, and Relevance to humans. Crit. Reviews in Toxicol. **29** (2), 169–261.

Roth B, Herkenrath P, Lehmann H-J, Ohles H-D, Hömig HJ, Benz-Bohm G, Kreuder J and Younossi-Hartenstein A (1988) Di(2-ethylhexyl) phthalate as plasticizer in PVC respiratory tubing systems: indications of hazardous effects on pulmonary function in mechanically ventilated, preterm infants. Eur. J. Pediatr. **147**, 41-46.

Rowland IR, Cottrell RC and Phillips JC (1977) Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. Fd. Cosmet. Toxicol. **15**, 17-21.

Rubin RJ and Schiffer CA (1976) Fate in humans of the plasticizer di-2-ethylhexyl phthalate, arising from transfusion of platelets stored in vinyl plastic bags. Transfusion **16** (4), 330-335.

Rubin RJ and Chang JCF (1978) Effects of the intravenous administration of the solubilized plasicizer di(2-ethylhexyl)phthalate on the lung and on survival of transfused rats (abstract). Toxicol. Appl. Pharmacol, **45**, 230.

Ruddick JA, Villeneuve DC, Chu I, Nestmann E and Miles D (1981) An assesment of the teratogenicity in the rat and mutagenicity in Salmonella of mono-2-ethylhexyl phthalate. Bull. Environ. Contam. Toxicol. **27**, 181-186.

Russel DJ and McDuffie (1986) Chemosphere **15** (8), 1003-1021.

Rykfors A-L (1998) Neste Oxo AB Sweden. Letter to KemI, 5.th June 1998.

Rykfors A-L (1997) Neste Oxo AB Sweden. Letter to KemI, 4.th June 1997

Sanchez JH, Abernethy DJ and Boreiko CJ (1987) Lack of di(2-ethylhexyl) phthalate activity in the C3H/10T½ cell transformation assay. Toxic. in Vitro **1**, 49-53.

Sanders HO, Mayer FL and Walsh DF (1973) Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environ. Res. **6**, 84-90.

Sanner T and Rivedal E (1985) Tests with the Syrian hamster embryo (SHE) cell transformation assay. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 665-671.

Sanner T, Mikalsen S-O and Rivedal E (1991) Hepatic peroxisome proliferators induce morphologic transformation of Syrian hamster embryo cells, but not peroxisomal β oxidation. **In**: Butterworth BE et al. (eds.). Progr. Clin. Biol. Res. **369**, 77-89.

Santillo D et al. (2003) Consuming Chemicals: Hazardous Chemicals in house dust as an indicator of chemical exposure in the home. Greenpeace Research Laboratories Technical Note 01/2003, April 2003, 74

Sasakawa S and Mitomi Y (1978) Di-(2-ethylhexyl)phthalate (DEHP) contentr of blood or blood components stored in plastic bags. Vox. Sang. **34**, 81-86.

Sax. Dangerous Properties of Industrial Materials.

Saxena DK, Srivastava SP, Chandra SV and Seth PK (1985) Testicular effects of di-(2-ethylthexyl) phthalate (DEHP): Histochemical and histopathological alterations. Ind. Health **23**, 191-198.

Schacht RA (1974) Pesticides in Illinois waters of Lake Michigan EPA Report No. PB 245 150.

Scheunert I, Vockel D, Schmitzer J and Korte F (1987), Chemosphere **16** (5), 1031-1041

Schilling K, Deckardt K, Gembardt Chr and Hildebrand B (1999) Di-2-ethylhexyl phthalate – two-generation reproduction toxicity range-finding study in Wistar rats. Continuos dietary administration. Department of Toxicology of BASF Aktiengesellschaft, D-67056 Ludwigshafen, FRG. Laboratory project idenfification 15R0491/97096. 511 pages. (referenced as BASF 15R0491/97096 in the IUCLID file)

Schilling K, Gembardt C and Hellwig J (2001) Di-2-ethylhexyl phthalate - Two-generation reproduction toxicity study in Wistar rats. Continous dietary administration. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen, FRG. Laboratory project identification 70R0491/97139. 1183 pages. (referenced as BASF 70R0491/97139 in the IUCLID file)

Schneider B, Schena J, Truog R, Jacobson M and Kevy S (1989) Exposure to di(2-ethylhexyl) phthalate in infants receiving extracorporal membrane oxygenation. N. Engl. J. Med. **320**, 1563.

Schmezer P, Pool BL, Klein RD, Komitowski D and Schmähl D (1988) Various short-term assays and two long-term studies with the plasticizer di-(2-ethylhexyl) phthalate in the Syrian golden hamster. Carcinogenesis **9**, 37-43.

Schmid P and Schlatter Ch (1985) Excretion and metabolism of di-(2-ethylhexyl) phthalate in man. Xenobiotica **15,** 251-256.

Schmitzer JL, Scheunert I and Korte F (1988) J. Agric. Food Chem. **36**, 210-215

Schoepe R and Pastuska G (1984) Alterung von PVC-Dachbahnen undter Kiesauflast, Kunststoffe im Bau **19**, 62-63.

Schoepe R and Pastuska G (1986) Langzeitverhalten handelsublicher Weich-PVC-Dachbahnen unter, Kuststoffe **76**, 272-274.

Schoepe R and Pastuska G (1987) Alterungsverhalten von von Dachbahnen aus Weich-PVC in abhängigkeit von der Materialdicke, Kuststoffe **77**, 693-695.

Scholz et al. (1998) Dietylhexylphthalate, Study on the determination of the bioaccumulation in carp (Cyprinus carpio) as specified by OECD 305. Hüls, Final report BA 006.

Scholz N (1994) Bestimmung der Auswirkungen von VESTINOL AH auf die Reproduktionsrate von Daphnia magna (nach OECD-Guideline 202 Teil II). Hüls Aktiengesellschaft, Abschlußbericht DL-160.

Scholz N (1995a) Determination of the effects of VESTINOL AH on the swimming behaviour of Daphnia magna (as specified by 92/69 EEC, Dec. 1992). Hüls Aktiengesellschaft,Final report DK-631.

Scholz N (1995b) Determination of the effects of VESTINOL AH on the reproduction rate of Daphnia magna (as specified by OECD Guideline 202 part 2). Hüls Aktiengesellschaft, Final report DL-160.

Scholz NJ (1998) Hüls AG in Marl. Personal communication.

Schuller HM and Ward JM (1984) Quantitative electron microscopic analysis of changes in peroxisomes and endoplasmic reticulum induced in mice during hepatocarcinogenesis by diethylnitrosamine promoted by di-(2-ethylhexyl) phthalate or phenobarbital. J. Exp. Pathol. **1**, 287-294.

Schulz CO, Rubin RJ and Hutchins GM (1975) Acute Lung toxicity and sudden death in rats following the iintravenous administration of the plasticizer, di(2-ethylhexyl)-phthalate, solubilzed with Tween surfactants. Toxicol. Appl. Pharmacol. **33**, 514.

Schweiger G, Sellner M, Golle B and Luttge U (1982) Effects of ecotoxicological chemicals on passive plasmalemma permeability in plants. Ecotoxicol. and Environ. Safety **7**, 366-372.

Schwetz BA (2001) JAMA **286**, 2085

Scott RC, Dugard PH, Ramsey JD and Rhodes C (1987) In Vitro Absorption of Some o-Phthalate Diesters Through human and Rat Skin. Plus Errata. Environ. Health Perspect. **74**, 223-227.

Seidl S, Gosda W and Repucci AJ (1991) The in vitro and in vivo evaluation of whole blood and red cell concentrates drawn on CPDA-1 and stored in a non-DEHP plasticized PVC container. Vox. Sang. **61**, 8-13.

Seth PK, Srivastava SP, Agarwal DK and Chandra SV (1976) Effects of di-2-ethyl-hexyl phthalate (DEHP) on rat gonads. Environ. Res. **12**, 131-138.

Shaffer CB, Carpenter CP and Smyth Jr HF (1945) Acute and subacute toxicity of di(2-ethylhexyl) phthalate with note upon its metabolism. J. Ind. Hyg. Toxicol. **27**, 130-135.

Shanker R, Ramakrishna C and Seth PK (1985) Environmental Pollution (Series A) **39** (1-7), 216-222

Sharman M, Read WA, Castle L and Gilbert J (1994) Levels of di-(2-ethylhexyl)phthalate and total phthalate esters in milk, cream, butter and cheese. Food Additives and Contaminants **11** (3), 375-385.

Sharpe RM, Walker M, Millar MR, Atanassova, Morris K, McKinnell C, Saundrs PTK and Fraser HM (2000) Effect of neonatal gonadotropin-releasing hormen antagonist administration on Sertoli cell number and testicular development in the marmoset: comparison with the rat. Biol. of Reprod. **62**, 1685-1693.

Sheldon L, Whitaker D, Keever J, Clayton A and Perrit R. (1993) Phthalates and PAHs in Indoor and Outdoor Air in a Southern California Community. Proceedings of Indoor Air ´93, 3.

Shell (1982) Bis(2-ethylhexyl) phthalate: Toxicokinetics of 14-day subacute oral administration to rats and marmosets. TSCATS: OTS 0539135, Doc. I. D.: 88-920002040, 05-21-82, Shell Oil Co.

Shell (1983) Induction of chromosome aberrations, polyploidy and sister chromatid exchanges in rat liver cells by chemical carcinogens. TSCATS: OTS 0515712, Doc. I. D.: 86-870001636, 10-01-83, Shell Oil Co.

Shibko and Blumenthal (1973) Toxicilogy of phthalic acid esters used in food-packaging material. Environ. Health Perspect. **4** (3), 131.

Shioda T and Wakabayashi M (2000) Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere **40**, 39-243.

Shimizu T, Kouketsu K, Morishima Y, Goto S, Hasegawa I, Kamiya TY and Kora S (1989) A new polyvinylchloride blood bag plasticized with less-leachable phthalate ester analogue, di-n-decyl phthalate, for storage of platelets. Transfusion **29**, 292-297.

Shiota K and Mima S (1985) Assessment of the teratogenicity of di(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate in mice. Arch. Toxicol. **56**, 263-266.

Shiota K and Nishimura H (1982) Teratogenicity of di(2-ethylhexyl) phthalate (DEPH) and di-*n*-butyl phthalate (DBP) in mice. Environ. Health Perspect. **45**, 65-70.

Shneider B, Schena J, Truog R, Jacobson M and Kevy S (1989) Exposure to di(2-ethylhexyl) phthalate in infants receiving extracorporal membrane oxygenation, N. Engl. J. Med. **320**, 1563.

Short RD, Robinson EC, Lington AW and Chin AE (1987) Metabolic and peroxisome proliferation studies with di-(2-ethylhexyl) phthalate in rats and monkeys. Toxicol. Ind. Health **3**, 185-195.

Siddall RA (1978) The use of marmosets (Callithrix jacchus) in teratological and toxicological research. Primates Med. **10**, 215-224.

Siddiqui A and Srivastava S (1992) Effects of di(2-hexyl) phthalate administration on rat sperm count and sperm metabolic enzymes. Bull. Environ. Toxicol. **48**, 115-119.

Silvo OEJ (1974) Alustavia turkimuksia dioktylftalaatin (DOP) kuutista myrkyllisyydestä kirjolohelle (Salmo gairdneri Richardson) ja sen vaikutuksista kasviplanktoniin sekä veden happitalouteen. Suomen kalatalous **47**, 19-25.

SIMPLETREAT chemical fate model for activated sludge sewage tratment plants. Wat. Sci. Tech. **33**, 279-287.

Singh HB et al. (1983) Reactivity/Volatility Classification of Selected Organic Chemicals: Existing Data and Measurement Protocols, EPA Cooperative Agreement CR810346-01, SRI Project 4954, U.S. Environmental Protection Agency, Research, Triangle Park, NC, USA

Singh AR, Lawrence WH and Autian I (1974) Mutagenic and antifertility sensitivities of mice to di(2-ethylhexyl) phthalate (DEHP) and demethoxyethyl phthalate (DMEF). Toxicol. Appl. Pharmacol. **29**, 35-46.

Singh AR, Lawrence WH and Autian J (1975) Maternal-foetal transfer of ^{14}C-di-(2-ethylhexyl) phthalate and ^{14}C-diethyl phthalate in rats. J. Pharm. Sci. **64,** 1347-1350.

Sittig M (1980) (Hrsg.): Priority toxic pollutants – health impoacts and allowable limits. Environmental Health Series No. 1. Noyes Data Corp. Park ridge, N.J, USA.

Sjöberg P, Bondesson U, Sedin G and Gustafsson J (1985a) Disposition of di- and mono-(2ethylhexyl)phthalate in newborn infants subjected to exchange transfusions.Eu. J. Clin. Invest. 15, 430-436.

Sjöberg PO, Bondesson UG, Sedin EG and Gustafsson JP (1985b) Exposure of newborn infants to plasticizers. Plasma levels of di-(2-ethylhexyl) phthal-ate and mono-(2-ethylhexyl) phthalate during exchange transfusion, Transfusion **25**, 424-428.

Sjöberg P, Bondesson U, Kjellen L, Lindquist N-G, Mentin G and Plöen L (1985c) Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. Acta Pharmacol. Toxicol. **56**, 30-37.

Sjöberg P, Bondesson U and Hammarlund M (1985d) Non-linearities in the pharmacokinetics of di(2-ethylhexyl) phthalate and metabolites in male rats. Arch. Toxicol. **58**, 72-77.

Sjöberg P, Lindquist NG, Montin G and Plöen L (1985e) Effects of repeated intravenous infusions of the plasticizer di-(2-ethylhexyl) phthalate in young male rats. Arch. Toxicol. **58**, 78-83.

Sjöberg P, Bondesson U, Gray TJB and Plöen L (1986a) Effects of di-(2-ethylhexyl) phthalate and five of its metabolites on rat testis *in vivo* and *in vitro*. Acta Pharmacol. Toxicol. **58**, 225-233.

Sjöberg P, Lindqvist NG and Plöen L (1986b) Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. Environ. Health Perspect. **65**, 237-242.

Skog P-J (1999) Letter from The City Real Estate Streets and Traffic Administration in the community of Stockholm 27 Oct. 1999.

Sloof W (1993) RIVM Report 670/92 LOC/EV Meetcampagne ftalaten tbv. scopingdocument ftalaten.

Smith-Oliver T and Butterworth BE (1987) Correlation of the carcinogenic potential of di(2-ethylhexyl) phthalate (DEHP) with induced hyperplasia rather than with genotoxic activity. Mutation Research **188**, 21-28.

Södergren A (1982) Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environm. Poll. (Series A) **27**, 263-274.

Solyom P and Ekengren Ö (1997) Begränsad ekotoxikologisk och kemisk undersökning av renat avloppsvatten från Neste Oxo in Stenungsund. Swedish Environmental Research Inst. IVL report 1997-12-11 (in Swedish).

Solyom P, Remberger M and Viktor T (2001) Further investigations on the influence of sediment associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog *Rana arvalis*. IVL report for ECPI/CEFIC.

Sorbe G (1984) Sicherheitstechnische Kenndaten Chemischer Stoffe; Ecomed, Landsberg, 1984; Loseblattsammlung, Stand 19, Erg-Lfg 10/90

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N and Serrano FO (1995) The E-SCREEN assay as a tool to identify oestrogen's: an update on estrogenic environmental pollutants. Environ. Health Perspect. **103**, 113-122.

Springborn Bionomics (1984a) Acute toxicity of thirteen phtalate esters to the sheepshead minnow (Cyprinodon variegatus). Report Number BP-84-2-14. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

Springborn bionomics (1984b) Acute toxicity of fourteen phthalate esters to Daphnia magna. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

Springborn bionomics (1984c) Chronic toxicity of fourteen phthalate esters to Daphnia magna. Toxicity test report submitted to Chemical Manufacturers Association, Washington, DC.

SRI INTERNATIONAL (1993) Speciality Chemicals. Advanced Ceramics Materials. Internal report. March 1993.

Srivastava S and Srivastava SP (1991) Effect of di(2-ethylhexyl) phthalate on 17β-hydroxy-steroid dehydrogenase activity in testis of rat. Toxicol. Letters **57**, 235-239.

Srivastava S, Awasthi VK, Srivastava SP and Seth PK (1989) Biochemical alterations in rat foetal liver following *in utero* exposure to di-(2-ethylhexyl) phthalate (DEHP). Indian J. Exp. Biol. **27**, 885-888.

Staples CA, Peterson DR, Parkerton TF and Adams WJ (1997). The Environmental Fate of Phtalate Esters. A Literature Review. Chemosphere **35**(4), 667-749.

Steele VE, Arnold JT, Arnold JV and Mass MJ (1989) Evaluation of a rat tracheal epithelial cell culture assay system to identify respiratory carcinogens. Environ. Mol. Mutagen. **14**, 48-54.

Steiner I, Kubesch K and Fiala F (1998) Migration of DEHP and DINP from PVC articles, preliminary summary. Austrian Standards Institute.

Sten K (1998) Personal communication. Rivners AB. Stockholm Sweden.

Stenchever MA, Allen MA, Jerominski L and Petersen RV (1976) Effects of bis(2-ethylhexyl) phthalate on chromosomes of human leukocytes and human foetal lung cells. J. Pharm. Sci. **65**, 1648-1651.

Stephenson RR (1982) The effects of water hardness, temperature, and size of test organism on the susceptibility of the freshwater shrimp, Gammarus pulex (L.) to toxicants. Shell Toxicology Laboratory report SBGR.82.085, experiment number 1816.

Stephenson RR (1983) Effects of water hardness, water temperature, and size of the test organism on the susceptibility of the freshwater shrimp, Gammarus pulex (L.), to toxicants. Bull. Environm. Contam. Toxicol. **31**, 459-466.

Stockholm vatten (1990) Avloppsvatten från hushåll. (In Swedish)

Stockholm vatten (1991a) Avloppsvatten från hushåll. Uppföljande undersökning 1991. (In Swedish)

Stockholm vatten (1991b) Avloppsvatten från Industrier. (In Swedish)

Streufert JM (1977) Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). A thesis presented to the faculty of the Graduate School University of Missouri - Columbia.

Streufert JM, Jones JR and Sanders HO (1980) Toxicity and biological effects of phthalate esters on midges (*Chironomus plumosus*). Transactions of the Missouri Academy of Science **14**, 33-40.

Strier MP (1977) US EPA Report.

Stringer R, Labunska I, Santillo D, Johnston P, Siddorn J and Stephenson A (1999)Concentrations of Phthalate Esters and Identification of Other Additives in PVC Children's Toys. ESPR-Environ. Sci.and Pollut.Res.6 (4) ??-?? (1999)

Styles JA, Clay P and Cross MF (1985) Assays for the induction of gene mutations at the thymidine kinase and the Na^+/K^+ ATPase loci in two different mouse lymphoma cell lines in culture. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 587-596.

Subba-Rao RV, Rubin HE and Alexander M (1982) Kinetics and extent of mineralisation of organic chemicals at trace levels in freshwater and sewage. Dept. of Agronomy, Cornell Univ., New York. Appl. and Environ. Microbiol. **43** (5), 1139-1150.

Sullivan KF, Atlas EL and Giam CS (1982) Adsorption of phthalic acid esters from seawater. Env. Sci. Technol. **16**, 428-432.

Swedish Environmental Protection Agency (1992) Slam. Innehåll av organiska miljöfarliga ämnen. Naturvårdsverket rapport 4085 (in Swedish).

Swedish Environmental Protection Agency. (1988) Organiska toxicka ämnen in slam från kommunala avloppsreningsverk .Naturvårdverket rapport 3514.

Tabak HH et al. (1981) JWPCF **53** (10), 1503-1518.

Takagi A, Sai K, Umemura T, Hasegawa R and Kurokawa Y (1992) Hepatomegaly is an early biomarker for hepatocarcinogenesis induced by peroxisome proliferators. J. Environ. Pathol. Toxicol. Oncol. **11**, 145-149.

Takagi A, Sai K, Umenura T, Hasegawa R and Kurokawa Y (1990) Relationship between hepatic peroxisome proliferation and 8-hydroxydeoxyguanosine formation in liver DNA of rats following long-term exposure to three peroxisome proliferators; di(2-ethylhexyl) phthalate, aluminium clofibrate and simfibrate. Cancer Letters **53**, 33-38.

Tamura H, Iida T, Watanabe T and Suga S (1990a) Long-term effects of peroxisome proliferators on the balance between hydrogen peroxide-generating and scavenging capacities in the liver of Fischer-344 rats. Toxicology **63**, 199-213.

Tamura H, Iida T, Watanabe T and Suga S (1990b) Long-term effects of hypolipidemic peroxisome proliferator administration on hepatic hydrogen peroxide metabolism in rats. Carcinogenesis **11**, 445-450.

Tamura H, Iida T, Watanabe T and Suga T (1991) Lack of induction of hepatic DNA damage on long-term administration of peroxisome proliferators in male F-344 rats. Toxicology **69**, 55-62.

Tanaka A, Adachi T, Takahashi T and Yamaha T (1975) Biochemical studies on phthalic esters. I. Elimination, distribution and metabolism of di-(2-ethylhexyl) phthalate in rats. Toxicol. **4**, 253-264.

Tandon R, Chowdhary SR, Seth PK and Srivastava SP (1990) Altered development of testis of rat exposed to di(2-ethylhexyl) phthalate (DEHP) during lactation. J. Environ. Biol. **11**, 345-354.

Tandon R, Parmar D, Singh GB, Seth PK and Srivastava SP (1992) The influence of low protein diet on the testicular toxicity of di(2-ethylhexyl) phthalate. Vet. Hum. Toxicol. **34**, 517-520.

Tandon R, Seth PK and Srivastava SP (1991) Effect of *in utero* exposure to di(2-ethylhexyl) phthalate on rat testes. Indian J. Exp. Biol. **26**, 1044-1046.

Tarkett-sommer AB (1999) Personal communication with Okmark P. and others (floor producer) 372 71 Ronneby Sweden.

Tarr BD, Barron MG and Hayton LH (1990) Effect of body size on the uptake and bioconcentration of Di-2-ethylhexyl phtalate in Rainbow trout. Washington state university, Pullman, Washington. Environ. Toxicol. Chem. **9**, (989-995).

Taylor BF, Curry RW and Corcoran EF (1981) Potetial for biodegradation of Phthalic Acid esters in marine regions. Appl. Environ. Microbiol. **42**, 590-595.

Teirlynck O, Kaufman JM, Bogaert MG and Roels H (1988) Testicular toxicity induced by single dosing of di- and mono-(2-ethylhexyl) phthalate in the rat. Toxicol. Lett. **40**, 85-91.

Tenneco (1981) 28 day hepatotoxicity study in rats. TSCATS: OTS 0510621, Doc. I. D.: 40-8165011, 12-22-81, Tenneco Chemicals.

Thiess AM, Korte A and Fleig H (1978a) Arbeitern mit Exposition gegenüber Di-2-ethylhexylphthalat (DOP). Verhdl. Dt. Ges. Arbeitsmed., Gentner Verlag Stuttgart, 137-154.

Thiess AM, Frentzel-Beyme R and Wieland R (1978b) Verhdl. Dt. Ges. Arbeitsmed., Gentner Verlag, Stuttgart, 155-164.

Thiess AM, Frentzel-Beyme R and Wieland R (1978c) Mortalitätsstudie bei Mitarbeitern mit exposition gegnüber Di-2-äyhylhexylphthalat (DOP).BASF, Aktiengesellschaft. 6700 Ludwigshafen/Rhein

Thiess AM and Fleig H (1978) Chromosomenuntersuchungen bei Mitarbeitern mit Exposition gegenüber Di-2-äethylhexylphthalat (DOP). Zbl. Arbeitsmed. **28**, 351-355.

Thinius K (1971) Stabilisierung und Alterung von Plastikwerk- stoffen 2, Akademie-Verlag, Berlin, (1971), zitiert nach: Rippen, Handbuch Umweltchemikalien, 13. Erg.Lfg. 1/92

Thölmann D (1996) Emission von Phthalaten (DEHP und DINP) aus Weich-PVC. Hüls AG, Zentrala Analytik, D-45764 Marl.

Thomas JA, Schein LG, Gupta PK, McCafferty RE, Felice PR and Donovan MP (1979) Failure of monoethylhexyl phtalate to cause teratogenic effects in offspring of rabbits. Toxicol. Appl. Pharmacol. **51** (3), 523-528.

Thomas JA, Wienckowski DB, Gillies BA, Thomas MJ and Youkilis EJ (1986) Effects of phthalic acid esters (paes) on the neonate and aspects of teratogenic actions. Environ. Health Perspect. **65**, 243-248.

Thompson RC, Stewart KM, Gillings E and Croudace C (1995) Di-2-ethylhexylphthalate and di-isodecyl phthalate: Effect on emergence of the midge, Chironomus riparius. Brixham Environmental Laboratory; ZENECA limited, Brixham, Devon TQ5 8BA, UK. Project Identification: X966/C, 19.

Thomsen M, Carlsen L And Hvidt S (2001) Solubilities and surface activities of phthalates investigated by surface tension measurements. Environ. Tox. Technol. **20** (1), 127-132.

Thurén A and Larsson P (1990) Environ. Sci. Technol. **24** (4), 554-559.

Thurén A and Woin P (1991) Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bull. Environm. Contam. Toxicol. **46**, 159-166.

Thurén A (1986) Determination of phthalates in aquatic environments. Bull. Environ. Contam. Toxicol. **36**, 33-40.

TNO (1996) PVC substance flow analysis for Sweden. Part 1. Main report. TNO-report STB/96/48-1.

Tomaszewski K, Agarwal DK and Melnick RL (1986) *In vitro* steady-state levels of hydrogen peroxide after exposure of male F344 rats and female B6C3F1 mice to hepatic peroxisome proliferators. Carcinogenesis **7**, 1871-1876.

Tomita I, Nakamura Y and Yagi I (1977) Phthlaic acid esters in various foodstuffs and biological materials. Ecotox. Environ. Safety **1**, 275-287.

Tomita I, Nakamura Y, Aoki N and Inui N (1982a) Mutagenic/carcinogenic potential of DEHP and MEHP. Environ. Health Perspect. **45**, 119-125.

Tomita I, Nakamura Y, Yagi Y and Tutikawa K (1982b) Teratogenicity/fetotoxicity of DEHP in mice. Environ. Health Perspect. **45**, 71-75.

Tomita I, Nakamura Y, Yagi Y and Tutikawa K (1986) Fetotoxic effects of mono-2-ethylhexyl phthalate (MEHP) in mice. Environ. Health Perspect. **65**, 249-254.

Topp E, Scheunert A, Attar A and Korte F (1986) Factors affecting the uptake of C-14-labeled organic chemicals by plants from soil. Ecotox. Environ. Safety **11**, 219-228.

Treinen KA and Heindel JJ (1992) Evidence that MEHP inhibits rat granulosa cell function by a protein kinase C-independent mechanism. Reprod. Toxicol. **6**, 143-148.

TSD (1991) Toxic Substances Division (TSD). Brooke, D.N. et al. (eds.) Environmental Hazard Assessment: Di-(2-ethylhexyl)phthalate, TSD/2.

Tsumura Y, Ishimitsu S, Saito I, Sakai H, Kobayashi Y and Tonogai Y (2001b) Eleven phthalate esters and di(2-ethylhexyl)adipate in one-week duplicate diet samples obtained from hospitals and their estimated daily intake. Food Addit. Contam. **18** (5), 449-460.

Tsumura Y, Ishimitsu S, Saito I, Sakai H, Kobayashi Y and Tonogai Y (2001a) Di(2-ethylhexyl)phthalate contamination of retailed packed lunch caused by PVC gloves used in the preparation of foods. Food Addit. Contam. **18** (6), 569-579.

Tsumura Y, Ishimitsu S, Saito I, Sakai H, Kobayashi Y and Tonogai Y (2003) Estimated daily intake of plasticizers in 1-week duplicate diet samples following regulation of DEHP-containing PVC-gloves in Japan. Food Addit. Contam. **20** (4), 317-324.

Tsutsui T, Watanabe E and Barrett JC (1993) Ability of peroxisome proliferators to induce cell transformation, chromosome aberrations and peroxisome proliferation in cultured Syrian hamster embryo cells. Carcinogenesis **14**, 611-618.

Tugwood JD, Aldridge TC, Lambe TG, MacDonald N and Woodyatt NJ (1996) Peroxisome proliferator activated-receptor: Structure and function. Ann. N.Y. Acad. Sci. **804**, 252-265.

Turnbull D and Rodricks JV (1985) Assessment of possible carcinogenic risk to humans resulting from exposure to di(2-ethylhexyl)phthalate (DEHP), J. Am. College Toxicol. **4**, 111-145.

Turnbull D and Rodricks JV (1989) A Comprehensive Risk Assesment of DEHP as a Component of Baby Pacifiers, Teehters, and Toys. The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies, John Wiley and Sons.

Turner JH, Petricciani JC, Crouch ML and Wenger S (1974) An evaluation of the effects of di(2-ethylhexyl) phthalate (DEHP) on mitotically capable cells in blood packs. Transfusion **14**, 560-566.

Tyl RW et al. (1992) The developmental toxicity of 2-ethylhexanol applied dermally to pregnant Fischer 344 rats. Fund. Appl. Toxicol. **19**, 176-185.

Tyl RW et al. (1991) Report No. RTI-307, Research Triangle Inst., Research Triangle Park, N.C., USA.

Tyl RW, Price CJ, Marr MC and Kimmel CA (1988) Developmental toxicity evaluation of dietary di(2-ethylhexyl) phthalate in Fischer 344 rats and CD-1 mice. Fundam. Appl. Toxicol. **10**, 395-412.

US EPA (1982) Bioconcentration kinetics of di-2-ethylhexylphthalate in fathead minnows, U.S. EPA/OPTS Public Files,Fiche #: OTS0206150, Doc#: 878210480

US EPA (US Environmental protection Agency) (1996) Exposure information for SIDS Initial Assessment in Non-Sponor Countries. Di(2-etylhexyl)Phthalate.

US EPA (US Environmental protection Agency) (1975) Report to congress, preliminary assessment of suspected carcinogens in drinking water. Office of Toxic Substaces.

UCD draft (1998) Draft "Use Category Document for plastic additives". Revised by the department of Environment (UK), Building Research Estabishment (BRE)

UKWIR (2004) UK Water Industry Research; Priority hazardous substances trace organics and diffuse pollution (Water Framework Directive), Urban catchment study and assessment of diffusive inputs; Report ref No 04/WW/17/3.

Umeda M, Noda K and Tanaka K (1985) Assays for inhibition of metabolic cooperation by a microassay method. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 619-622.

Umweltbundesamt (1987) (Hrsg.) UBA-Jahresbericht 1987. Berlin.

Union Carbide (1950) One-year doses of plasticizer DOP to guinea pigs. TSCATS: 206059, Doc. I. D.: 878212206, 02-28-50, Union Carbide Corp. and TSCATS: 206150, Doc. I. D.: 878211381, 02-28-50, Dow Chemical Co.

Union Carbide (1951) Chronic toxicity of plasticizer DOP (one-year oral doses to dogs). TSCATS: 206059, Doc. I. D.: 878212207, 20-01-51, Union Carbide Corp.

Uno Y, Takasawa H, Miyagawa M, Inoue Y, Murata T and Yoshikawa K (1994) An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. Mutation Research **320**, 189-205.

US CDC (US Centers for Disease Control and Prevention), National Center for Environmental Health (2003) Second National Report on Human Exposure to Environmental Chemicals, Department of Health and Human Services, Division of Laboratory Sciences Atlanta, Georgia 30341-3724, NCEH Pub. No. 02-0716 (www.cdc.gov/exposurereport/phthalates).

v.Pöhner A, Simrock S, Thumulla J, Weber S and Wirkner T. Hintergrundbelastung des Hausstaubes von Privathaushalten mit mittel- und schwerflüchtigen organischen Schadstoffen. Umwelt und Gesundheit 2/97 (1997)

Vainiotalo S and Pfäffli P (1990) Air Impurities in the PVC Plastics Processing Industry. Ann. occup. Hyg. **34** (6), 585-590.

Vanden Heuvel JP (1999) Forum: Peroxisome Proliferator-Activated Receptors (PPARS) and Carcinogenesis. Toxicol. Sciences **47**, 1-8.

Vapour pressure versus 1/T plots of plasticisers, solvents etc from "The Technology of Plasticisers" by Sears and Darby.

Varga F and Csaky TZ (1976) Changes in the blood supply of the gastrointestinal tract in rats with age. Pflugers Arch. Jul 30 **364** (2), 129-343.

Varga F et al. (1975) Effects of gastric emptying rate on the intestinal absorption of chloroquine in rats. Pharmacol. **13** (5), 401-8.

Venkataramanan R and Hurckart GJ et al. (1986) Leaching of Di. Am. J. Hosp. Pharm. **43**, 2800-2802.

Vessman J and Rietz G (1974) Determination of di(ethylhexyl) phthalate in human plasma and plasma proteins by electron capture gas chromatography,Chromtatogr. **100**, 153-163.

Versar Inc. (1980) Human exposure to di(2-ethylhexyl)phthalate. Final Report Contract 68-01-5791. Submitted to Assessment Division, Office of Pesticides and Toxic Substances, U.S. Environmental protection Agency, Washington DC.

Vercheuren K (1983) Handbook of Environmental Data on Organic Chemicals; Van Nostrand Reinhold, New York; 2nd ed. 1983.

Vikelsö J, Thomasen M and Johansen E (1998) Sources of phthalates and nonylphenoles in municipal waste water. A study in a local environment. NERI Technical Report No. 225.

Vikelsöe J, Thomsen M, Johansen E and Carlsen L (1999) Phthalates and Nonylphenols in soil. Ministry of Environment and Energy, National Environmental Rersearch Institute. NERI Technical Report No. 268.

Vikelsøe J, Jensen GH, Johansen E, Carlsen L and Rastogi SC (1997) Migration af Phthalater fra bideringe. Afdelning for Miljøkemi, Danmarks Miljøudersøgelser, Roskilde.

Villeneuve DC, Franklin CA, Chu I, Yagminas A, Marino IA, Ritter L and Ruddick JA (1978) Toxicity studies on mono-2-ethylhexyl phthalate. Toxicol. Appl. Pharmacol. **45**, 250-251.

Vogel EW (1985) The Drosophila somatic recombination and mutation assay (SRM) using th *white-coral* somatic eye color system. **In**: Ashby, J and de Serres FJ (eds), Elsevier Sci. Publ. **5**, 313-317.

Vollebregt LHM, van der Ven MCE and Rijs GBJ (1997) Microverontreinigigen in toiletpapier. RIZA-werkdocument: 97.141X, Lelystad, november 1997 *(only in dutch).*

Volskay VT and Leslie Grady CP (1988) Toxicity af selected RCRA compounds to activated sludge microorganisms. J. Water. Pollut. Control Fed. **60**, 1850-1856.

Waddell WJ, Marlowe C, Miripol JE and Garvin PJ (1977) The distribution in mice of intravenously administered plasma solutions of (^{14}C)di(2-ethylhexyl) phthalate determined by whole-body autoradiography. Toxicol. Appl. Pharmacol. **39**, 339-353.

Waggy GT (1972) Surfactants and plasticizers completeness of biodegradation in a simulated river system. Union Carbide Corporation. Project no.: 122H01/215E15. File No.:16668. US-EPA report 878212202 / OTS 84003A.

Waggy GT and Payne JR (1974) Environmental impact product analysis acute toxicty testing. Union Carbide Corporation. Project no.: 910F44. File No.:19133

Waldock MJ (1983) Determination of phthalate esthers in samples from the marine environment using GC-MS. Chem. in Ecol. **1**, 261.

Wams TJ (1987) Diethylhexylphthalate as an environmental contaminant – a review. Sci.Total Environ. **66**, 1-16.

Ward JM, Rice JM, Creasia D and Riggs C (1983a) Dissimilar patterns of promotion by di-(2-ethylhexyl) phthalate and phenobarbital of hepatocellular neoplasia initiated by diethylnitrosamine in B6C3F1 mice. Carcinogenesis **4**, 1021-1029.

Ward JM and Reynolds CW (1983b) Large granular lymphocyte leukemia, a heterogeneous lymphocyte leukemia in F-344 rats. Am. J. Pathol. **111**, 1-10.

Ward JM, Hagiwara A, Anderson LM, Lindsey K and Diwan BA (1988) The chronic hepatic or renal toxicity of di(2-ethylhexyl) phthalate, acetaminophen, sodium barbital, and phenobarbital in male B6C3F1 mice: autoradiographic, immunohistochemical, and biochemical evidence for levels of DNA synthesis not associated with carcinogenesis or tumor promotion. Toxicol. Appl. Pharmacol. **96**, 494-506.

Ward JM, Konishi N and Diwan BA (1990) Renal tubular cell or hepatocyte hyperplasia is not associated with tumor promotion by di-(2-ethylhexyl) phthalate in B6C3F1 mice after pransplacental initiation with N-nitrosoethylurea. Exp. Pathol. **40**, 125-138.

Ward JM, Ohshima M, Lynch P and Riggs C (1984) Di-(2-ethylhexyl) phthalate but not phenobarbital promotes N-nitrosodiethylamine-initiated hepatocellular proliferative lesions after short-term exposure in male B6C3F1 mice. Cancer Lett. **24**, 49-55.

Ward JM, Peters JM, Perella CM and Gonzalez FJ (1998) Receptor and nonreceptor-mediated organ-specific toxicity of Di-(2-ethylhexyl) phthalate (DEHP) in peroxisome proliferator-activated receptorα-null mice. Toxicol. Pathol. **26** (2), 240-246.

Warren JR, Lalwani ND and Reddy JK (1982) Phthalate esters as peroxosome proliferator carcinogens. Environ. Health Persp. **45**, 35-40.

Watari N, Kanai M, Torizawa K, Mabuchi Y and Hida Y (1978) Electron microscopical and cytochemical observations of the mouse liver following the dministration of phthalate ester (DOP). J. Clin. Electron Microscopy **11**, 103-120.

Webb JS, van der Mel HC, Nixon M, Eastwood JM, Greenhalgh M, Read SJ, Robson GD and Handley PS (1999) Plasticizers Increase Adhesion of the Deteriogenic Fungus *Aureobasidium pullulans* to Polyvinyl Chloride. Am. Soc. for Microbiol. **65** (8), 3575-3581.

Webber MD and Lesage S (1989) Waste Management and Research **7**, 63-82.

Webber MD and Nichols JA (1995) Waste Water Techn. Centre (WTC), operated by Rockcliffe Reseach Management Inc. Febr. 1995.

Webber MD and Roger HR, Watts CD, Boxall ABA, Davis RD and Scoffin R (1996) The Sci. of the total Environ. **185**, 27-44.

Webber MD and Wang C (1995) Industrial organic compounds in seleced Canadian soils. Can. J. of Soil Sci. 513-524.

Weghorst CM, Devor DE, Henneman JR and Ward JM (1993/1994) Promotion of hepatocellular foci and adenomas by di-(2-ethylhexyl) phthalate and phenobarbital in C3H/HeNCr mice following exposure to N-nitrosodiethylamine at 15 days of age. Exp. Toxic. Pathol. **45**, 423-431.

Welsch-Pausch K, McLachlan MS and Umlauf G (1995) Determination of the principal pathways of polychlorinated dibenzo-p-doxines and dibenzofurans to Lolium multiflorum (Welsh Ray Grass). Environ. Sci. Technol. **29**, 1090-1098.

Wennberg L et al. (1996) The Infuence of Phtalate Esters (DEHP and DIDP) on Hatching of frog Eggs and Survival of Tadpoles (Moorfrog, Rana arvalis). IVL-draft report.

Wennberg L, Parkman H, Remberger M, Viktor T and Williams C (1997) The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. IVL, Box 21060, S-100 31 Stockholm, Sweden. IVL report B1260, 26.

Wester RC, Melendres J, Sedrik L, Maibach H and Riviere JE (1998) Percutaneous Absorption of Salicylic Acid, Theophylline, 2,4-Dimethylamine, Diethyl Hexyl Phthalic Acid, and p-Aminobenzoic Acid in the Isolated Perfused Porcine Skin Flap Compared to Man in Vivo. Toxicol. Appl. Pharmacol. **151**, 159-165.

Whalan JE and Pettigrew HM (1997) Inhalation Risk assessments and the Combining of Margins of Exposure. Health effects Division. Office of pesticide Products. United States Environmental Protection Agency.

Whalberg C (1997) Stockholm Vatten MI. Personal communication 1997-09-09.

White RD, Carter DE, Earnest D and Mueller J (1980) Absorption and metabolism of three phthalate esters by the rat small intestine. Fd. Cosmet. Toxicol. **18**, 383-386.

WHO (1998) GRMS/Food international dietry survey: Infant exposure to certain organochlorine contaminants fron breast milk-A risk assessment. Food Safety Issues. WHO/FSF/FOS/ Geneva, Switzerland.

WHO, International Programme on Chemical Safety (IPCS), (1992). Diethylhexyl phthalate. Environmental Health Criteria 131. World Health Organization, Geneva.

Wildbrett G (1977) Difusion of phthalic acid esters from PVC milk tubing. Environ. Health Perspect. **3**, 29.

Williams MD, Adams WJ and Parkerton TF (1995) Sediment Sorption Coefficient Measurements for four Phthalate Esters: Experimental Results and Model Theory. Env. Tox. Chem. **4** (9), 1477-1486.

Williams DT (1973) Dibutyl- and Di-(2-ethylhexyl)phthalate in fish. J. Agr. Food. Chem. **21**, 1128-1129.

Williams GM, Maruyama H and Tanaka T (1987) Lack of rapid initiating, promoting or sequential syncarcinogenic effects of di-(2-ethylhexyl) phthalate in rat liver carcinogenesis. Carcinogenesis **8**, 875-880.

Williams GM, Tong C and Brat SV (1985) Tests with the rat hepatocyte primary culture/DNA-repair test. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 341-345.

Wilson AS, Biggin IS and Pugh DM (1978) The influence of volatility on the selection of plasticisers to meet new developing performance requirements (BP Chemicals Limited). PVC Processing, Int. Conf.

Wilson WB, Giam CS, Goodwin TE, Aldrich A, Casrpenter V and Hrung YC (1978) The toxicity of phthalates to the marine dinoflagellate Gymnodinium breve. Bull. Environm. Contam. Toxicol. **20**, 149-154.

Wofford HW, Wilsey CD, Neff GS, Giam CS and Neff JM (1981) Bioaccumulation and metabolism of phthalate esters by Oysters, Brown shrimp, and Sheepshead minnows. Dept. of Biol. and Dept. of Chem., Texas A and M Univ., Texas. Ecotoxicol. and Environ. Safety **5** (202-210)

Woggon H and Koehler U (1967) The testing of plastics consumer goods. Polarographic determination of bis(2-ethylhexyl) phthalate and study of its migration tendecy (trans). Kunststoffe **57**, 583.

Woin P and Larsson P (1987) Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bull. Environ. Contam. Toxicol. **38**, 220-225.

Wolfe et al. (2003) Multigeneration reproduction toxicity study in rats (unaudited draft): Diethylhexylphtalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet. TherImmune Research Corporation (Gaithersburg, Maryland), TRC Study No 7244-200. (http://ntp.niehs.nih.gov/go/15182).

Wolfe NL (1985) Screening of Hydrolytic Reactivity of OSW Chemicals, Report of Environmental Research Laboratory, Athens, GA 30613, USA, for the Office of Solid Waste, U.S. EPA, (1985) (zitiert nach: Rippen, Handbuch Umweltchemikalien, 13 Erg.Lfg.1/92.

Wolfe NL, Burns LA and Steen WC (1980b) Chemosphere **9**, 393-402.

Woodward KN (1988) Phthalate esters: Toxicity and metabolism. Vol I. CRC Press, Boca Raton (Florida).

Woodyatt NJ, Lambe KG, Myers KA, Tugwood JD and Robert RA (1999) The peroxisome proliferator (PP) response element upstream of the human acyl CoA oxidase gene is inactive among a sample human population: significance for species differences in response to PPs. Carcinogenesis. **20**, 369-372.

Würgler FE, Graf U and Frei H (1985) Somatic mutation and recombination test in wings of *Drosophila*. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 325-340.

Yagi Y, Nakamura Y, Tomita I, Tsuchikawa K and Shimoi N (1980) Teratogenic potential of di- and mono-(2-ethylhexyl) phthalate in mice. J. Environ. Pathol. Toxicol. **4**, 533-544.

Yoon JS, Mason JM, Valencia R, Woodruff RC and Zimmering S (1985) Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. Environ. Mutagen. **7**, 349-367.

Yoshikawa K, Tanaka A, Yamaha T and Kurata H (1983) Mutagenicity study of nine monoalkyl phthalates and a dialkyl phthalate using *Salmonella typhimurium* and *Escherichia coli*. Fd. Chem. Toxicol. **21**, 221-223.

Yoshioka Y, Ose Y and Sato T (1986) Correlation of the five test methods to assess chemical toxicity and relation to physical properties. Ecotoxicology and Environmental Safety **12**, 15-21.

Yoshioka Y, Ose Y, Goto M and Hikino H (1987) Relations between the test methods for ecotoxicity. Eisei Kagaku **33** (1), 11-19.

Younoszai MK and Ranshaw J (1973) Gastrointestinal growth in the fetus and suckling rat pups: effects of maternal dietary protein. J. Nutr. Mar. **103** (3), 454-61.

Youssef J and Badr M (1998) Extraperoxisomal targets of peroxisome proliferators: mitochondrial, microsomal, and cytosolic effects. Implications for health and disease. Critical Reviews in Toxicol. **28** (1), 1-33.

Zacharewski TR et al. (1998) Examination of the *In Vitro* and *In Vivo* Estrogenic Activities of Eight Commercial Phthalate Esters. Accepted for publication May 19, 1998. Toxicol. Sci. **45**

Zaebst D, Elliot LJ and Donaldson HM. In-Depth Industrial Hygiene Survey Report of Hickory Manufacturing Company, Hickory, North Carolina, Report No IWS-74-10.

Zaroogian GE et al. (1985) Environ. Toxicol. and Chem. **4**, 3-12.

Zeiger E, Haworth S, Mortelmans K and Speck W (1985a) Mutagenicity testing of di(2-ethylhexyl) phthalate and related chemicals in *Salmonella*. Environ. Mutagen. **7**, 213-232.

Zeiger E and Haworth S (1985b) Tests with a preincubation modification of the Salmonella/microsome assay. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 187-199.

Zeiger E, Haworth S, Speck W and Mortelmans K (1982) Phthalate ester testing in the National Toxicology Program's environmental mutagenesis test development program. Environ. Health Perspect. **45**, 99-101.

Zetzsch C (1991). UWSF - Z.Umweltchem. Oekotox. **3** (1), 59-64.

Zimmering S, Mason JM and Valencia R (1989) Chemical mutagnesis testing in Drosophila VII. Results of 22 coded compounds tested in larval feeding experiments. Environ. Mol. Mutagen. **14**, 245-251.

Zimmermann FK, Heinisch J and Scheel I (1985) Tests for the induction of mitotic aneuploidy in the yeast *Saccharomyces cerevisiae* strain D61.M. **In**: Ashby, J and de Serres FJ (eds), Elsevier Science Publ. **5**, 235-242.

Zitko V (1972) Determination, toxicity, and environmental levels of phthalate plasticisers, Fisheries research board of Canada, Technical Report No. 344.

Zitko V (1973) Determination of phthalates in environmental samples. Internatl. J. Environ. Anal. Chem. **2**, 241.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECDIN	Environmental Chemicals Data and Information Network
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives

JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
o/oo	Parts per thousand
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent

PAH	Polycyclic aromatic hydrocarbons
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SCHER	Scientific Committee on Health and Environmental Risks
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand

UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

European Commission

EUR 23384 EN **European Union Risk Assessment Report**
bis(2-ethylhexyl) phthalate (DEHP), **Volume 80**

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The report provides the comprehensive risk assessment of the substance bis(2-etylhexyl)phthalate (DEHP). It has been prepared by Sweden in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I - Environment

This part of the evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment concludes that there is concern for the aquatic and terrestrial ecosystems in generic scenarios arising from the use of DEHP in production of lacquers, paints, printing inks, sealants, or adhesives, and at industrial sites processing polymers containing DEHP.

There is at present no concern for the atmosphere or micro-organisms in sewage treatment plants.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is no concern for risks from the physico-chemical properties of DEHP. For workers, children exposed to DEHP-containing toys and child-care articles, children and adults undergoing some medical treatments using medical equipment containing DEHP, and for children eating food grown locally near industrial sites handling DEHP, there is concern for effects on testis, kidney, fertility, and for developmental toxicity.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission’s committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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