ANNEX XV RESTRICTION REPORT

PROPOSAL FOR A RESTRICTION

SUBSTANCE NAME: BIS(2-EHTYLHEXYL)PHTHLATE (DEHP), BENZYL BUTYL PHTHALATE (BBP), DIBUTYL PHTHALATE (DBP), DIISOBUTYL PHTHALATE (DIBP)

IUPAC NAME: Bis(2-ethylhexyl) phthalate
EC NUMBER: 204-211-0
CAS NUMBER: 117-81-7

IUPAC NAME: Benzyl butyl phthalate
EC NUMBER: 201-622-7
CAS NUMBER: 85-68-7

IUPAC NAME: Dibutyl phthalate
EC NUMBER: 201-557-4
CAS NUMBER: 84-74-2

IUPAC NAME: Diisobutyl phthalate
EC NUMBER: 201-553-2
CAS NUMBER: 84-69-5

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CONTENT

PROPOSAL FOR A RESTRICTION

A. Proposal

This proposal addresses the risks posed by the four already classified (Rep cat. 1B) phthalates DEHP, BBP, DBP and DIBP. These substances are all reported to affect testicular functions and to have adverse effects on sexual differentiation during the developmental process. They are furthermore found to exert anti-androgenic effects. These toxicological effects of the four phthalates have raised concerns regarding their endocrine-disrupting chemical properties in terms of reproductive and developmental disorders in humans. In order to reduce these effects the dossier addresses the combined exposure from the four phthalates from articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes.

Existing legal requirements and risk management measures
DEHP, DBP and BBP have been included in REACH Annex XIV. Use or placing on the market for a use of these phthalates are therefore subject to authorisation according to the procedures in Title VII of REACH. Placing on the market of articles containing these phtalates is, however, not covered by the authorisation procedure and thus the authorisation process does not cover imported articles. Numerous articles could therefore still contain the four phthalates.

DEHP, DBP and BBP are also subject to restrictions in REACH Annex XVII, entry 51. According to entry 51 DEHP, DBP and BBP shall not be used as substances or in mixtures in toys and childcare articles in a concentration greater than 0.1 % by weight of the plasticised material. Furthermore toys and childcare articles containing these phthalates in a concentraion greater than 0.1 % by weight of the plasticised material shall not be placed on the market.

With effect as from 20 July, 2013 the Toy Safety Directive (2009/48/EC) will ban the use of substances that are classified as carcinogenic, mutagenic or toxic to reproduction (CMR) of category 1A, 1B or 2 under Regulation (EC) No 1272/2008 in toys, in components of toys or in micro-structurally distinct parts of toys in individual concentrations above the specific limit for classification. DEHP, DBP, BBP and DIBP have all been classified as toxic to reproduction of category 1B.

In addition there are a number of internal market directives which in more general terms address health aspects of the articles which they regulate. Examples of such directives are the Construction Products Directive (89/106/EEC) which is to be replaced by a Regulation, the General Product Safety Directive (2001/95/EC) and the Machinery Directive (2006/42/EC).

Widespread use
Phthalates are found primarily in PVC as softeners but can also be found in other plastics in low concentrations. Phthalates can also be found in e.g. dispersions, paints and varnishes, as emulsifiers, repellents and carrier fluids in biocides, in cosmetics and perfumes.

Due to the use of phthalates in articles in the indoor environment as e.g. vinyl floorings, wall paper, furniture and other consumer articles (see chapter B.2), dust and indoor air are among the main sources for phthalate exposure.

Due to the widespread use, concern is raised regarding human exposure to phthalates in consumer articles, as phthalates in articles will contribute both to the exposure from the indoor environment, but eventually also to the exposure via food. The exposure of phthalates via food is originating from environmental pollution, and the environmental pollution could originate from production of articles, the use of articles and when articles end up in the waste stream.

Groups of population in focus
The focus in this dossier and thus in the calculations and simulations of exposure estimates has been on 2-year-olds (15.2 kg bw), 6/7-year-old (23.1 kg bw) and for adults (60 kg bw), as representatives of the wide span of the general population.

The group of 2-year-olds has been selected as they are particularly susceptible due to their physical size, proportions (large surface/small volume), stage of development and behavior. This group plays with almost anything they can get their hands on. Their activity level is relatively high. They still have a residual of mouhting behavior left and many of their activities are still down on the floor. The group can therefore be expected to have a relatively high exposure of phthalates because of their behaviour.

6/7-year olds are selected as they may be expected to have contact with a great number of articles. The exposure is in most cases not expected to be by oral contact, but rather by dermal contact. Single articles may though be expected to be mouthed as for example erasers.

Adults are chosen as the last group of population in focus. Adults will as the other groups of the population be exposed to phthalates through food, indoor environment and articles. The exposure is though expected to be lower due to their higher body weight and a different behaviour compared to 2-years old and 6/7-years old. Even though children are the most sensitive group the exposure and risk of adults should also be under control.

Exposure
Individuals are exposed to phthalates through inhalation (phthalates emitted from wall paper, floor covering and other sources), ingestion (via e.g. food, dust and toddlers sucking on plastic materials), and dermal exposure for their whole lifetime, since the intrauterine life.

Individuals can be exposed through dust and air from the indoor environment. The level of phthalates in the indoor environment will depend on the number and volumes of products containing phthalates. In a study from 2005 of the dust levels of children's rooms in Sweden, 52 % of the houses had PVC flooring in the child's bedroom, and BBP and DEHP levels in dust were significantly higher (2- and 1.2 fold, respectively) in rooms with PVC flooring than in those without PVC. It is not known whether this use is common in all Europe but it is known that significant amounts of the phthalates are used for this application. Furthermore, phthalates have a tendency to redistribute from its initial location to all indoor surfaces when it is introduced into an indoor environment, and they can persist indoors for years after they are introduced, even after the primary source is removed.

The exposure from indoor environment is calculated both based on data from the literature where the concentration(s) of phthalates are measured in dust, and also based on modelled data on the concentration(s) of the phthalates in the air.

Exposure can also take place through direct contact with articles. For adults this would primarily be through dermal contact, while sucking and ingestion also should be taken into consideration when the exposure of children is calculated. The exposure from articles is calculated based on analyses of the content and migration of the phthalates. On this background realistic worst case scenarios are made.

Phthalates have been used in food contact materials, but the use in food contact material is restricted today. The exposure from food is calculated based on data from the literature. Unfortunately only rather old data on this source of the phthalates can be found, and it can be expected that the level of phthalates in food would be lower today, because one of the major sources of phthalates in food was considered to be from food contact materials, which are regulated today. Food is though still expected to be one of the sources to phthalate exposure and one study has shown phthalates in food packed in recycled paperboard, collected in 2009. Phthalates in food can also be found due to the fact that phthalates from the environment can end up in food. There are no new informations of the size of this source, however. US EPA states, that there are limited data on the migration of phthalate from plasticized PVC into environmental media. However, due the total volume of plasticized PVC produced, it is possible that PVC or other polymer/polymericlike materials containing phthalates may be long-term and dispersive sources of human and environmental exposures to phthalates (US EPA 2009).

Furthermore the exposure of the four phthalates is calculated based on biomonitoring data. These data will give a picture of the real exposure the population has been exposed to of the four phthalates, as the biomonitoring data are measurements of phthalate metabolites in urine from humans.

Dose addition

In Chapter E.3.5 Step 5, in the process of risk characterisation according to REACH guidance (Part E: Risk characteriation) special attention to combined exposures is addressed. Combined exposures may emerge in situations where the same person is potentially exposed to the same substance in the same setting via different routes of entry into the body or from different products containing the same substance. In cases, where exposure occurs to several very closely related and similar acting substances, the exposure evaluation and risk characterisation should reflect this aspect. The most relevant way of reflecting this is by introducing the method of dose addition.

DEHP, DBP BBP and DIBP are all already classified as toxic to reproduction (Repr. 1B) and are all considered to be endocrine disruptors (anti-androgen effect, see further in B 5.9). Furthermore, EU Risk Assessments have already been made for DEHP, DBP and BBP individually.

Due to their similar mode of action in combination with new scientific knowledge, it is both reasonable and a necessity to introduce dose addition as a new method to get a more realistic picture of the risks related to the four phthalates. Dose addition calculation is a method to predict the combination effects and is also often referred to as concentration addition, simple similar action or Loewe additivity. The additive effects are described mathematically by summing up the doses of the individual chemicals in a mixture adjusted for their differences in potencies. This is further described in section B.1.4.2.

There is a close agreement between findings from the literature (Howdeshell et al., 2008 and 2007, Christiansen et.al., 2009, Rider et.al., 2009 and Kortenkamp & Faust, 2010) and the conclusions from an expert workshop held in Denmark in 2009 (Kortenkamp & Hass, 2009) suggesting that the dose addition models adequately describe the effects of mixtures of endocrine disrupting chemicals with both similar and dissimilar modes of action. DEHP, DBP, BBP and DIBP belong to the group of substances with similar mode of action.

At the request of the US EPA, the US National Academy of Sciences committee on cumulative risk assessment of phthalates and related chemicals has been established. The report of this committee was made publicly available in December 2008 and in the summary of this report is stated: "*Thus, the evidence supports the use of dose-addition as an approximation in estimating cumulative risk posed by phthalates and other anti-androgens. The use of a dose-addition model is also supported by data that show cumulative effects at doses at which individual mixture components did not induce observable effects*" and moreover; "*Cumulative risk assessment based on common adverse outcomes is a feasible and physiologically relevant approach to the evaluation of the multiplicity of human exposures*". Hence the use of dose addition as a method to assess the combined exposure to DEHP, BBP, DBP and DIBP can be justified. For further justification see section B.1.4.

Operational conditions and risk management measures are not under control
It is demonstrated in this restriction dossier (B 9.3.2) that the combined exposure to DEHP, BBP, DBP and DIBP from food, dust and indoor air combined with normal handling and use of a few selected articles containing one or several of these phthalates reaches levels that constitute a risk to children. A comparison with biomonitoring data of urine metabolites further confirms that risk.

This restriction dossier includes the results from test of only a few articles. There could therefore still be numerous of articles containing DEHP, BBP, DBP and DIBP available to the general population on the market. Several reports indicate that single articles (containing high concentrations of phthalates) may be responsible for the major contribution to a very high exposure to phthalates. This implies that the regulatory measures taken so far in REACH and other existing legal requirements (see section B.9.1.1) is not sufficient to eliminate or to reduce the total exposure to DEHP, BBP, DBP and DIBP to a safe level.

There is therefore a concern that the total exposure to these four phthalates from all sources together may constitute a serious risk to the human health. Even though a high exposure to the phthalates may be due to exposure to phthalates from single articles it is essential to include all other articles contributing to the concentration of phthalates in dust and indoor air in order to address the problem in an effective manner. The major contributors to the concentration of the four phthalates found in the indoor environment in the gas phase, particles in the air and dust are the articles like e.g. furniture, toys, PVC flooring and wall paper.

It is also important to note that the data show that the risk is not sufficiently controlled for a large part of the population. There is therefore a need to reduce the exposure from the four phthalates. Phthalates in the food does not originate from the food processing, as this is already strictly regulated, but must be due to environmental pollution. This pollution may stem from the production of articles, use of the articles or when the articles end up in the waste stream. The concentration of the four phthalates in the indoor environment is originating from the articles containing these phthalates found in the indoor environment. A regulation of the four phthalates in articles in the indoor environment and articles with direct contact may therefore be expected to reduce the

exposure to the indoor environment in the short term as well as the exposure from food in the long term.

A.1 Proposed restriction(s)

A.1.1 The identity of the substance(s)

- Chemical Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester **(DEHP)**
- IUPAC Name: Bis(2-ethylhexyl) phthalate
- EC Number: 204-211-0
- CAS Number: 117-81-7

- Chemical Name: Benzyl butyl phthalate **(BBP)**
- IUPAC Name: Benzyl butyl phthalate
- EC Number: 201-622-7
- CAS Number: 85-68-7

- Chemical Name: Dibutyl phthalate **(DBP)**
- IUPAC Name: Dibutyl phthalate
- EC Number: 201-557-4
- CAS Number: 84-74-2

- Chemical Name: Diisobutyl phthalate **(DIBP)**
- IUPAC Name: Bis(2-methylpropyl) benzene-1,2-dicarboxylate
- EC Number: 201-553-2
- CAS Number: 84-69-5

A.1.2 Scope and conditions of restriction(s)

As mentioned in section A above, it is essential to include all sources of phthalates – be it from articles contributing to the concentration in the indoor environment or from articles which are in direct contact with skin or mucous membranes – when considering a proposal for a restriction which takes the combined exposure of the phthalates in question into account.

This poses a challenge when defining the scope of the restriction. Various alternatives have been considered and rejected (see section E). Ultimately the wording in the restriction proposed below was decided upon, the reason being that it appropriately covers the range of articles that should be comprised by the restriction when taking combined exposure into account.

Based on the justifications summarised in Section A.2 and discussed in the report, the following restrictions with derogations are proposed for the placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes:

For DEHP, DBP, BBP and DIBP a ban is proposed on the placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes

containing one or more of these phthalates in a concentration greater than 0.1 % by weight of any plasticised material.

For these substances it is furthermore proposed that:

- the restriction includes a transition period enabling the market to adjust. The transition period should take depletion of stocks into account. As for the length of this transition period a balance must be struck between the need for protecting human health and the possibility for the market to adjust (e.g. 12 months from the date of entry into force of the restriction).
- by way of derogation, the ban should not apply to the immediate packaging of medicinal products covered by Regulation (EC) No 726/2004, Directive 2001/82/EC or Directive 2001/83/EC, or to medical devices covered by Directive 90/385/EEC, Directive 93/42/EEC or Directive 98/79/EC.
- by way of derogation, the ban should not apply to toys. Toys containing DEHP, DBP and BBP are covered by an existing restriction. Futhermore with effect from 20. July, 2013 Directive 2009/48/EC on the Safety of Toys will ban the use of substances that are classified as carcinogenic, mutagenic or toxic for reproduction (CMR) of category 1A, 1B or 2 under Regulation (EC) No 1272/2008 in toys, in components of toys or micro-structurally distinct parts of toys unless certain specified conditions are met.
- By way of derogation, the ban should not apply to childcare articles in respect of DEHP, DBP and BBP. Childcare articles containing DEHP, DBP and BBP are covered by an existing restriction.
- by way of derogation, the ban should not apply to articles intended to come into contact with food covered by Regulation (EC) No 1935/2004 and specific measures under this regulation, e.g. Commission Regulation (EU) No 10/2011.
- by way of derogation, the ban should not apply to articles intended for use indoors and articles which may come into direct contact with the skin or mucous membranes which were in use in the European Union prior to the date of entry into force of the restriction.
- the restriction includes a definition of ‘childcare article’ corresponding to the definition in the existing restrictions in entries 51 and 52 in Annex XVII
- the term “use” is defined in the restriction as meaning any placing, keeping, storing, hanging, laying, applying, mounting, fixing or other application indoors of articles.

<u>Discussion of the scope and conditions:</u>

The proposal is to ban the placing on the market of articles intended for use indoors in unsealed applications and articles that may come into direct contact with the skin or mucous membranes containing one or more of the 4 phthalates DEHP, DBB, BBP or DIBP in a concentration greater than 0.1 % by weight of any plasticised material.

The wording “in a concentration greater than 0.1 % by weight of any plasticised material” was chosen as this would be easy to enforce and covers the general thinking behind the proposal and as the phthalates are only used as plasticisers this could be clearly addressed in the proposal. Another wording could be “articles or any parts thereof” which would also cover the intentions that no part should contain any of the 4 phthalates as this would contribute to the exposure.

Like most other restrictions concerning placing on the market of articles containing one or more specified chemicals there will be borderline cases. This could also be the case for this restriction depending on the actual wording of the restriction that the Commission may propose

Examples of articles that are covered by this proposal, but are not limited to, are:
- childcare articles (for DIBP)
- interiors of cars, trains, ships, boats, aircrafts etc.
- wall covering and flooring
- insulation on wires used indoors
- insulation on cables used indoors in unsealed applications
- coated fabric and film/sheets used for furniture
- coated fabric and film/sheets used for bags and briefcases/suitcases and similar items
- coated fabrics and film/sheets used for tablecloth, curtains, shower curtains and similar items
- carpet tiles/squares produced with foam as back cover
- water mattresses and air mattresses
- wallpaper/tapestry
- footwear
- textiles
- bathing equipment (swim jackets, wings, belts and pools - inflatable and others)
- erasers
- balance balls for playing (not toys) and physical exercises
- sex toys

The restriction on phthalates does in our eyes not comprise a.o.:
- insulation on wires and cables used outdoors
- large-scale stationary industrial tools
- roofing material
- car undercoating
- garden tools which only have phthalates contained in other parts than handles.

As for other restrictions not implying a total ban on all articles containing one or more substances the proposed restriction will imply some uncertainty on border cases. Even this could be avoided, if all articles were included disregarding their lack of contribution to the human exposure; however, this is not justified as the restriction is based on the combined exposure that gives rise to concern.

Some cables intended for outdoor use could also be used indoors. For such products an easy and cheap solution could be labelling, where the label could specify that the article is only intended for outdoor use or something like that. Cables to be built in walls etc. where no migration to the indoor environment is expected is not included in the scope. The installation process it self may imply some direct skin contact but this is assumed to be performed by professionels who can wear gloves in order to reduce the risk.

Garden hoses are handled and the direct skin contact is the reason for the garden hoses to be included in the restriction.

The restriction includes a definition of the term “use” as meaning any placing, keeping, storing, hanging, laying, applying, mounting, fixing or other application indoors of articles. This has the consequence that those articles still allowed to be placed on the market have to be stored outdoors.

For all four substances it is proposed that immediate packaging of medicinal products, medical devices and articles intended to come into contact with food are exempted from the ban. For these product categories it is assumed the risk is adequately addressed through the relevant legislations.

The phthalates can be used in paints and lacquers. These products are however not included in this proposal as such products are mixtures and not articles. According to Article 58(5) in REACH, after inclusion of a substance in Annex XIV a substance shall not be subject to new restrictions under the procedure outlined in Title VIII covering the risks to human health or the environment from the use of the substance on its own, in a mixture or incorporation of a substance in an article arising from the intrinsic properties specified in Annex XIV. However, with reference til Art. 58(6) (and that is the legal background for this proposal) there is a derogation concerning substances listed in Annex XIV covering the risks to human health or the environment from the presence of the substance in (an) article(s).

This means that paints and lacquers with at least the 3 of the 4 phthalates are covered by the Authorisation procedure.

Besides this, due to the classification as Rep cat. 1B and the inclusion on Annex VI in the REACH Regulation it is not allowed to sell mixtures with the four phthalates to the general public according to Annex XVII, 30 in the REACH Regulation. Only professionals are therefore allowed to buy paints and laquers containing one or more of the four phthalates and this reduces the use considerably. Recent data from the Danish Product Register shows that there is a use of DEHP in paints and laquers, but the use is very well below 100 kg per year. This is in contrast to the estimated amounts in the whole EU in 2007 which for this use estimated to be 900 tonnes per year (see section B.2.2.12).

Following is an example wording for a restriction based on the above. In order better to differentiate between the existing and the proposed new restriction for DEHP, DBP and BBP the example is shown as a new entry (51a) for these substances.

Example:

51a. The following phthalates (or other CAS and EC numbers covering the substance): (a) Bis (2-ethylhexyl) phthalate (DEHP) CAS No 117-81-7 EC No 204-211-0 (b) Dibutyl phthalate (DBP) CAS No 84-74-2 EC No 201-557-4 (c) Benzyl butyl phthalate (BBP) CAS No 85-68-7 EC No 201-622-7 (d) Diisobytyl phthalate (DIBP) CAS No 84-69-5 EC No 201-553-2	1. Articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing one or more of these phthalates in a concentration greater than 0.1 % by weight of any plasticised material shall not be placed on the market after (12 months from entry into force). 2. By way of derogation, paragraph 1 shall not apply to the immediate packaging of medicinal products covered under Regulation (EC) No 726/2004, Directive 2001/82/EC or Directive 2001/83/EC, or to medical devices covered under Directive 90/385/EEC, Directive 93/42/EEC or Directive 98/79/EC. 3. By way of derogation, paragraph 1 shall not apply to toys. By way of derogation, paragraph 1 shall not apply to childcare articles as regards DEHP, DBP and BBP. 4. By way of derogation, paragraph 1 shall not apply to articles intended to come into contact with food covered by Regulation (EC) No 1935/2004 and specific measures under this regulation, e.g. Commission Regulation (EU) No 10/2011. 5. By way of derogation, paragraph 1 shall not apply to articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes that were in use in the European Union before (date of entry into force). 6. For the purpose of this entry 'childcare article' shall mean any article intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children. 'Use' shall mean any placing, keeping, storing, hanging, laying, applying, mounting, fixing or other application indoors of articles.

A.2 Summary of the justification

A.2.1 Identified hazard and risk

The four phthalates have a very widespread and dispersive use. They are found in a great variety of articles from floor coverings to sandals. Due to this widespread use a concern is

raised regarding human exposure to phthalates in articles. Individuals are exposed to phthalates through inhalation (phthalates emitted from wall paper, floor covering and other sources), ingestion (via e.g. food, children mouthing plastic materials), and dermal exposure for their whole lifetime, since the intrauterine life.

The phthalates DEHP, DBP, BBP and DIBP are reported to affect testicular functions and to have adverse effects on sexual differentiation during the developmental process. They are furthermore found to exert anti-androgenic effects. These toxicological effects of the four phthalates have raised concerns regarding their endocrine-disrupting chemical properties in terms of reproductive and developmental disorders in humans.

The four phthalates are all classified as reprotoxic category 1B according to Regulation (EC) No 1272/2008 (CLP Regulation) and reprotoxic cat. 2 accoding to Directive 67/548/EEC. These effects have been discussed for other phthalates but they are not classified so far; this proposal is therefore only concentrated on phthalates where there are agreement on the reprotoxic effects. For some of the alternatives studies show this effect (DINP), but the reported potency are significantly lower than for the four phthalates (NOAEL around 300 mg/kg bw/d in comparison to N(L)OAELs varying from 2-125 mg/kg bw/d. It is reckognised that some is questioning the studies regarding the reprotoxic effects of DINP.

Overall, the selected DNELs for these phthalates (DEHP 25 µg/kg bw/day; DBP 6.7 µg/kg bw/day; BBP 500 µg/kg bw/day; DIBP 1250 µg/kg bw/day) based on NOAELs for anti-androgenic effects in developmental studies are considered the most relevant available data of the current risk assessment, as dose addition is used to calculated the risk.

The exposure and risk is calculated for three population groups (2-year old, 6/7 years old and adults) for articles, indoor environment and food, respectively. To calculate the risk a risk characterization ratio (RCR) is calculated as a total RCR for all the four phthalates. If the RCR is above 1 the risk is not controlled. A RCR value is calculated for a worst case scenario/95th percentile, high median exposure and the lowest median exposure. The 95th percentile estimates are considered to be "realistic worst case exposure" levels. The "high median" exposure estimates were selected as high median value of exposure to each phthalate from each source. In order to make a scenario representing lower exposures, we selected the lowest median value of each phthalate from each source.

Exposure and risk from articles
In three projects from 2010 the Danish EPA has demonstrated that the four phthalates can be found in several and diverse articles. Mainly articles contributing to the exposure of children to phthalates, either via direct contact or indirectly via indoor air, have been brought into focus. The content and migrations of the four phthalates in the articles, to which children and adults are directly exposed to as well as other articles, have been measured and used in the exposure assessment.

The migration from especially three individual types of articles resulted in extensive exposure to humans: sandals, sex toys and erasers.

It should be noted, that the high exposure from sandals to adults is due to a high migration of phthalate measured from a single pair of plastic sandals.

The use of sex toys can lead to a very high exposure as some sex toys have shown to contain and emit very high concentrations of phthalates. The migration will to a high extent also depend on the use of an oil based lubricant. The migration of DEHP has shown to increase with a factor of more than 900 with the use of an oil based lubricant.

The possible intake of erasers has specifically been calculated for children in the early school-age (6/7- year olds). The calculation of exposure to phthalates from erasers (high estimate) was based on intake of 8 mg eraser per day (corresponding to the weight of 2-3 sesame seeds per day). The RCR from intake of eraser based on mouthing for 1 hour/day is calculated to be 0.63 for lowest and highest median exposure estimate. If it is assumed that the eraser is only mouthed 10 minutes per day the RCR contribution from erasers would be 0.1. For realistic worst case level the scenario of eating 8 mg is chosen, giving a RCR of 7.

In Table 1 the calculated RCR values for median and realistic worst case exposure can be seen.

Table 1. Calculated RCR values for articles.

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95^{th} percentiles or max)**
RCR articles	**2-year old** **6/7-year old** **Adult**	0.066 0.74 0.19	0.066 0.74 0.19	0.18 7.6 1.6

RCR values for articles show that the risk is not controlled in worst case situations for 6/7-year olds and for adults. This picture is opposite to "the normal situation", where it could be expected that the highest risk is for the 2-years old. The opposite picture demonstrated here is because of the exposure from single articles giving a high exposure as the sandals and sex toys for adults and the erasers for 6/7-year olds.

There are uncertainties when these estimations of the risk from articles are made. It is for example assumed that the same adult is using sex toys and the plastic sandal with the highest migration of phthalates, and that this person is using all the articles in the worst case scenario. This will overestimate the risk from articles, especially in the worst case scenario. It could be expected that some persons use some articles as set up in the median exposure scenarios and some articles as set out in the worst case scenario, and the risk could be somewhere between these two scenarios.

However, it should be noted that the specific articles included in these exposure estimates represent only a few of numerous sources of phthalate exposure from articles. For that reason, the cumulative exposure may easily reach levels of serious concern and a need of further control and limitation of the exposure to the four phthalates, especially for children.

Exposure and risk via indoor environment

Phthalates have the capacity to persist indoors for years after they are introduced, and even after the primary source is removed. Phthalates are not chemically bound to the polymer matrix of e.g. vinyl flooring, and slow emission from the articles to air or other media usually occurs during the entire use phase of the articles. If the surface materials contain phthalates and the only mechanism of removal is normal ventilation, it is impossible to avoid the phthalates in indoor air, and the substances can persist indoors for thousands of hours to many years. High-frequent cleaning of all indoor surfaces (incl. walls etc.) will possible reduce the concentration of phthalates in the air.

Exposure from the indoor environment can happen via indoor air or via dust. The exposure via air is calculated with three different approaches.

The indoor air levels of the phthalates were simulated by the use of data from furniture/materials marketed in Denmark. By applying the simulation it was found that DEHP is by far the dominating phthalate in indoor air. For that reason, a calculation has also been made by applying the method referred to in the EU Risk Assessment Report on DEHP (EU RAR, 2008). Finally, these levels have been compared to air levels of DEHP found in the literature.

The total concentration of phthalates in air (phthalate vapours and phthalates associated to particles) was calculated to be approximately 4.8 resp. 1 µg/m^3 in a realistic worst case resp. realistic scenario. Based on these levels, RCR is calculated (Table 2).

The exposure from dust is calculated based on literature data and RCR values are calculated and added to the RCR values for indoor air as shown in Table 2.

Table 2 RCR values for indoor environment.

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95th percentiles or max)**
RCR indoor air	**2-year old** **6/7-year old** **Adult**	0.01 0.012 0.002	0.01 0.012 0.002	0.05 0.06 0.01
RCR dust	**2-year old** **6/7-year old** **Adult**	0.11 0.072 0.009	0.35 0.23 0.032	1.6 1.1 0.14
RCR indoor environment (air and dust)	**2-year old** **6/7-year old** **Adult**	0.12 0.084 0.011	0.36 0.24 0.034	1.65 1.16 0.15

The RCR values for indoor environment shows that the risk in not controlled for the 95th percentiles for 2-year olds and 6/7-year olds with RCR values of 1.65 and 1.16 respectively, while the risk is under control for the median exposure (RCR: 0.03-0.36).

Phthalates in the indoor environment originates from the articles in the indoor environment (e.g. vinyl flooring, balance balls and furniture). The concentration will for example depend on:

- the concentration of phthalates contained in the articles
- the emission rate from the surface of the articles
- physical activities in a room (whirling dust around)
- ventilation rates
- room temperature
- the level and frequency of cleaning
- numbers and volume of sinks (sorptive reservoirs) in the room.

A reduction of the use of phthalates has effects on the indoor air concentration levels of phthalates as seen in e.g. the simulated scenarios. There is a redistribution of phthalates going on via the air from the sources to all other surfaces in a room, including human skin, clothes, etc. The content in air may have consequences for building up phthalate levels in the indoor environment on surfaces and dust which then further contribute to the indirect exposure.

Thus there are arguments for the control and limiting of the sources that generate concentrations of phthalates in the indoor environment.

Exposure and risk from food
The exposure of the four phthalates via food is calculated based on literature data. There are though only older data on the levels of phthalates, and these data do not encompass a voluntary agreement of the phasing out of phthalates in tubes for milk and foils for food, that earlier on were one of the large exposure pathways. Furthermore EU legislation on phthalates in food contact materials entered into force in 2008 and this is expected to lower the exposure of phthalates from food. An exposure of phthalates from food is though still expected, as one other source of phthalates in food is from environmental pollution. Environmental pollution comes from the production of articles, articles used out door and from the articles ending as waste.

There are no new studies to confirm lower levels of the four phthalates in food today and the measurements of the exposure of the four phthalates in food are therefore based on the available data. The calculated exposure of the four phthalates from food will therefore most probably be overestimated but one study with data from 2009 shows a migration of phthalates in infant food packed in recycled paperboard.

The data used are data from opinions from EFSA (2005) on DEHP, DBP and BBP for use in food contact materials and these data are based on phthalate intake from food from two Danish studies, based on dietary measurements.

Table 3 below shows the RCR values calculated from exposure via food for the four phthalates.

Table 3. RCR values for food.

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95^{th} percentiles or max)**
RCR food	**2-year old**	0.24	2.2	4.2

	6/7-year old **Adult**	0.17 0.078	0.97 0.33	2.1 1.8

Based on the available data on food it may be concluded, that these results strongly indicate the need for further control and limitation of the exposure to phthalates, especially for 2-year olds, as the RCR is above one from the exposure from food alone, based on "high median" RCR (RCR 2.2). RCR for 6/7-year olds from food alone is very close to 1 (0.97) for "high median". It should though

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95th percentiles or max)**
RCR biomonitoring	**Child** **Adult**	**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

be emphasized that these calculations are based on old data and that the exposure must be expected to have decreased. There are though no available data that indicate the level of exposure from food today. US EPA states, that there are limited data on the migration of phthalate from plasticized PVC into environmental media. However, due the total volume of plasticized PVC produced, it is possible that PVC or other polymer/polymericlike materials containing phthalates may be long-term and dispersive sources of human and environmental exposures to phthalates (US EPA 2009).

Exposure and risk based on biomonitoring data

The main part of the studies are performed on adult humans, a few studies are made on children older than 6 years and only one study presents data on 2 to 4 year olds. The biomonitoring data are measured before the legislation of phthalates in food contact materials entered into force in 2008, but after the voluntary agreement on the phasing out of phthalates in tubes for milk and foils for food. The data are also from before the EU ban of some phthalates in toys and childcare articles from 2007 (REACH Regulation annex XVII). Before the EU ban of phthalates in 2007, an emergency ban on 6 phthalates in toys and childcare articles for children less than 3 years of age were already in force. Even though legislation has entered into force after the measurements of the biomonitoring data, attention were thus already on phthalates at the time of the measurements.

Table 4 below shows RCR values on biomonitoring.

Table 4. RCRs based on biomonitoring data

As can be seen from the results in table 4 it is only the RCRs based on the lowest median, that where lower than 1 (0.6) for children. Even RCRs based on high median are inclining that there is a risk that is not sufficiently controlled for children.

Cumulative risk assessment

Table 5 below the sum of the RCR values can be seen and compared to the RCR values based on biomonitoring data.

Table 5. Total RCR values and RCR values based on biomonitoring data.

	Age	RCR for "lowest median" exposure estimates	RCR for "high median" exposure estimates	RCR for realistic worst case scenario (95^{th} percentiles or max)
RCR indoor environment and articles	**2-year old** **6/7-year old** **Adult**	**0.19** **0.82** **0.20**	**0.43** **0.98** **0.22**	**1.83** **8.76** **1.75**
RCR total (indoor environment, articles and food)	**2-year old** **6/7-year old** **Adult**	**0.43** **1.0** **0.28**	**2.7** **1.9** **0.55**	**6.1** **11** **3.6**
RCR biomonitoring	**Child** **Adult**	**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

The sum of the median RCR values for articles and indoor environment are lower than the RCR values based on biomonitoring data for all three age groups. This indicates that the difference between these is from the exposure from food. If the same comparison is made for the 95^{th} percentiles, then the sum of the RCR values for articles and indoor environment are higher than the RCR values based on biomonitoring data. This could be because of an overestimation of the exposure from articles in the worst case scenario as it is assumed that one person is using more than one article in a worst case scenario.

Comparing and adding the RCR values for the articles, indoor environment and food (RCR total) strongly indicate that the exposure of the four phthalates could give rise to a risk for anti-androgenic effects among exposed individuals following cumulative exposure to the four phthalates for these age groups. Even though they have different potency their similar mode of action makes it reasonable to perform dose addition (see B.1.4.2) to predict the overall exposure and the combination effects of these substances. In a worst case scenario the total RCR values indicate a risk to all population groups and even though the total calculated RCR values (articles, indoor environment and food) seem to be overestimated especially for the articles and food, the RCR for biomonitoring data also show values above 1 for the 95^{th} percentile.

The cumulative approach to this risk assessment makes it clear that even with relatively low background phthalate exposures (such as the "low median" values for children) giving an RCR below 1, an added exposure from articles to any one of the four phthalates in sufficiently high amounts may result in an increase of the RCR above 1 and thus, identify an increase in the risk for anti-androgenic effects among exposed individuals.

The biomonitoring data acts as supportive evidence of the risk posed to cumulative exposure to the phthalates as it is only the RCRs based on the lowest median, that where lower than 1 (0.6) for children. Even RCRs based on high median are inclining that there is a risk that is not sufficiently controlled for children.

It should be noted that there are uncertainties associated with both DNELs and exposure estimates and therefore, RCR values just above 1 should be interpreted bearing these uncertainties in mind. However, the approach using "low median", "high median" and "realistic worst case" scenarios for the RCR calculations is an attempt to investigate the consequences of the uncertainty in exposure assessment by evaluating how much the total RCR changes when background exposure estimates change. RCR values just below 1 should also be interpreted cautiously because the RCR values only include considerations of the four specific phthalates. Thus cumulative risk assessment including also other anti-androgenic compounds than the four phthalates may therefore lead to even higher RCR values.

Phthalates in the indoor environment and in food have been estimated to be a significant source to the exposure of phthalates even though there are uncertainties in the estimations and the assumptions made. Phthalates in the indoor environment are originating from the articles present indoor, as these articles will emit phthalates to the air and dust in the indoor environment. Phthalates found in food are originating from environmental pollution. This environmental pollution can come from articles used outdoor, the production of articles or when the articles end up as waste. The main sources to the exposure of phthalates are therefore from the articles used and the articles having a direct contact to skin or mucous membranes.

The data show that for a large part of the population the risk is not sufficiently controlled and the exposure to DEHP, DBP, BBP and DIBP should be reduced.

A.2.2 Justification that action is required on a Community-wide basis

As explained in section B, the hazard properties of the four phthalates are widely recognized as they are classified as Rep. Cat 1B according to the CLP Regulation.

As also reported in Section B, the four phthalates are used in a variety of consumer articles throughout the EU. Consequently, emissions originating from the articles during their indoor use, take place in all the Member States, even though the emissions in different parts of the EU may vary depending on the status of the switch to articles with alternatives to the four phthalates due to awareness of both consumers and producers/importers and perhaps different uses of specific types of articles due to e.g. climate conditions.

Therefore, the risks need to be controlled on a Community-wide basis.

A.2.3 Justification that the proposed restriction is the most appropriate Community-wide measure

Only one type of restriction will address the risks posed by the combined effect of the four phthalates: The placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing one or more of these phthalates in a concentration greater than 0.1 % by weight of any plasticised material. The reason is

that the relevant risk management option (RMO) should address exposures caused by emissions from all articles that emit the four phthalates to the indoor environment as well as from articles where there is a direct exposure to humans. DEHP, DBP and BBP are included in Annex XIV of the REACH Regulation and are thus subject to the authorisation process. However, the authorisation process does not cover any imported articles. Numerous articles could therefore still contain the four phthalates. Other types of RMOs like voluntary agreements, labelling etc. do not address this properly as demonstrated in section E.3.1.

Furthermore as the costs related to substitution to other plasticisers are considered to be relatively small, only an RMO option on all article types used indoor and direct contact is considered in the proposal.

Besides, this type of restriction sets out a level playing field for all stakeholders and gives both producers and importers the same obligations, and in this respect takes care of differentiation between importers and producers within the authorisation process.

The proposed restriction will result in a significantly decreased exposure of the public to the four phthalates. The decrease in exposure will not be measurable from the first day after the entry into force of a restriction. The exposure of phthalates from dust will continue as long as there are products emitting phthalates in the indoor environment and there will be exposure from food as the phthalates may still be found in the environment in a long period after a restriction has entered into force. On the long term the exposure from these sources will also decline as a result of the proposed restriction.

In section B.2.2.1–B.2.2.12 the following article groups were selected for getting information on the use of the 4 phthalates, use of alternatives and the financial implications for the use of alternatives.

- Flooring (and heavy wall covering)
- Insulation on wires and cables
- Electronic devices
- Plast coated fabric and film/sheets used for bags and brief/suitcases and similar items
- Plast coated fabrics and film/sheets used for tablecloth, curtains, shower curtains and similar items (not industrial uses)
- Carpet tiles/squares produced with (typically) PVC-foam as back cover
- Water- and air mattresses
- Plast coated wallpaper/tapestry
- Footwear
- Bathing equipment (swim-coats/wings/belts and pools - inflatable and others)
- Balls for training and physical exercises
- Others: Erasing rubber

The reasons for choosing these article groups for this type of study were several. It was known that these articles either produced or imported potentially could contain the 4 phthalates. It was also known that substitution had already taken place for some of the articles at least to a certain extend

and that looking further into these could give information on alternatives and related financial implications. Besides, some of the producers were well known from previous studies conducted by either the consultant or by the Danish EPA. It should also be mentioned that these groups were generally the groups that the Danish EPA knew of in this respect at the onset of the preparation of the dossier that contributed to the exposure in respect to the targeted effect.

The dossier submitter had hoped for information on uses in other articles /article groups during the work with the dossier, e.g. through the many contacts with industry and importers. This was however not the case, and the expected information in the registration dossiers on uses in individual articles was not given in (any) sufficient detail.

One obvious challenge in getting information on articles with the 4 phthalates and socio-economic effects of a ban is the lack of knowledge for imported articles that only enters the European market on a "day-to-day" basis, typically different cheap articles made of PVC sold by "direct sale" retailers. These articles could be any kind of articles but they are impossible to define either as a group or individually.

The article groups discussed in section B.2.2 cover a broad range of different articles. As mentioned before, the dossier submitter has no information on other actual uses, but as the groups are so widely dispersed and covering the broad range other unknown remaining uses could be expected to be within the range of the already treated article groups.

A supportive argument is that the treated article groups constitute the vast majority of the amounts used for articles within the EU. The figures are however a bit difficult to estimate precisely due to the changes in the import/production in the registration dossiers compared to the estimated amounts given in the ECHA reports from 2009 (Echa 2009 a-d) on "Data on manufacture, import, export, uses and releases of the 4 different phthalates" and the figures calculated in the report on the individual article groups on which the data presented in B.2.2.1-B.2.2.12 are based (Danish EPA 2011).

Alternatives

The existence of alternatives for all applications underlines the possibilities for a quick phase-out. The alternatives available may include other phthalates, plasticisers that are not phthalates as well as other types of materials. No examples have been identified of products for which alternatives are not available.

The dominant alternatives to the four phthalates reported used for the covered product groups are DINP and DIDP. For most applications, however, non-orthophthalate plasticisers such as DOTP, and other plasticiser types such as DINCH, ASE, ATBC, COMGHA and dibenzoates are actually in use, or being considered by producers as realistic alternatives to ortho-phthalates.

As stated above, the preferred alternative to the four phthalates for a large part of the articles is so far DINP. It has to be underlined that some experimental data indicates a potential for having the same characteristics as the four phthalates (Repr. Cat. 1B). Studies showing that DINP has anti-androgenic effects on reproductive development, all show that DINP is less potent than DEHP,

DBP, DIBP and BBP[1] and regarding effects on human health DINP would therefore be a better alternative than the four phthalates proposed to be regulated.

As shown in Section C.13.1-13.3 with a comparison made in section C.14.1-3 the alternatives have better environmental and toxicological profiles compared to the four phthalates on most endpoints. It can be concluded that the overall assessment of the alternatives shows, that they do not pose an additional risk compared to the 4 phthalates DEHP, DBP, BBP and DIBP. For some of the endpoints (other than reprotoxicity) some of the alternatives do not seem better than the 4 phthalates, but since non are classified and the effects of the most used phthalates are less significant compared to the 4 phthalates it can be concluded, that using the alternatives instead of the 4 phthalates in question will result in an overall benefit.

Costs

Substitution of classified phthalates with DINP/DIDP seems generally to be the least expensive option, as these phthalates for most applications can substitute directly for the four phthalates with no major changes needed in the production process. The effective relative price of DINP (reflecting both the price per kg and the need for more plasticiser to obtain the same effect) was in 2006 about 11% higher than the price of DEHP. The relative price of DIDP was about 21% higher. As the concentration of plasticisers in the polymer matrix can be up to 40% of the product by weight, the price of the alternatives will influence the price of such final products. However, the cost of plasticisers comprises only a minor part of the total production price of the article as demonstrated in section E.2.1.2.

The proposal for restriction concerns the placing on the market of articles containing one or more of the four phthalates. In general, no problems related to the implementability have been detected as shown in section E.2.1.3. The data available seems to indicate that in those cases the four phthalates are eliminated by substitution to other plasticisers, costs of raw materials will normally be the dominant cost element to be considered, while cost elements like a change of manufacturing process and changes in production equipment seems to be of less significance for the products groups covered.

No information has been received that substitution of the four phthalates should imply major implementation problems, even though some changes in processing and material composition may require some research and development, but the R&D cost of substituting e.g. DINP for DEHP is assumed to be relatively low as shown in section E.2.1.3.

The proposed restriction is considered to be manageable. Three of the four phthalates are already restricted in other products (toys and childcare articles) with the same content limit.

The placing on the market of articles containing one or more of the four phthalates can be enforced mainly by controlling producers, importers or retailers.

Extra costs are estimated to be related to control by the manufacturers, importers and the authorities. The presence of the four phthalates cannot be determined by simple screening methods, e.g. XRF; therefore sampling, extraction and laboratory analysis are required.

[1] Values for NOAEL (LOAEL for DBP) are given in Table 15 and C.2.2.6.

The price of an analysis of DEHP, DBP and BBP in a flexible PVC is in Denmark reported to be about 190 € per sample when more than 20 samples are analysed.

The testing cost is relevant for enforcement authorities and for importers in cases where these want to ensure that the imported articles are in compliance. However in practice the importer, retailers etc would normally rely on information from suppliers on the content.

B. Information on hazard and risk

B.1 Identity of the substance(s) and physical and chemical properties

This proposal concerns four phthalates with similar modes of action. Even though the phthalates have different potency their similar modes of action makes it reasonable to perform dose addition calculations to predict the combination effects of these chemicals.

B.1.1 Name and other identifiers of the substances

Chemical Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (**DEHP**)
IUPAC Name: Bis(2-ethylhexyl) phthalate
EC Number: 204-211-0
CAS Number: 117-81-7

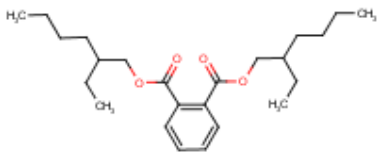
Chemical Name: Benzyl butyl phthalate (**BBP**)
IUPAC Name: Benzyl butyl phthalate
EC Number: 201-622-7
CAS Number: 85-68-7

Chemical Name: Dibutyl phthalate (**DBP**)
IUPAC Name: Dibutyl phthalate
EC Number: 201-557-4
CAS Number: 84-74-2

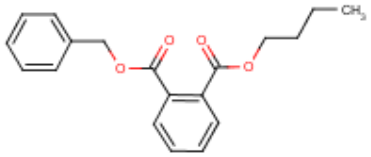
Chemical Name: Diisobutyl phthalate (**DIBP**)
IUPAC Name: Bis(2-methylpropyl) benzene-1,2-dicarboxylate
EC Number: 201-553-2
CAS Number: 84-69-5

B.1.2 Composition of the substance(s)

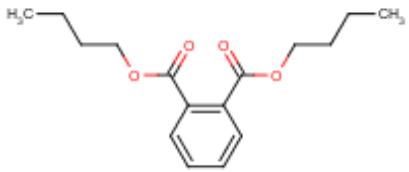
Chemical Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (**DEHP**)
Molecular weight: 390.6 g/mol
Molecular formula: C24H38O4
Structural formula:



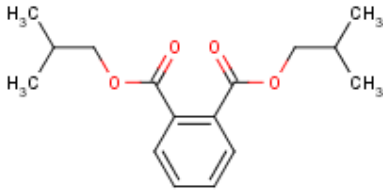
Chemical Name: Benzyl butyl phthalate (**BBP**)
Molecular weight: 312.35 g/mol
Molecular formula: C19H20O4
Structural formula:



Chemical Name: Dibutyl phthalate (**DBP**)
Molecular weight: 278.34 g/mol
Molecular formula: C16H22O4
Structural formula:



Chemical Name: Diisobutyl phthalate (**DIBP**)
Molecular weight: 278.34 g/mol
Molecular formula: C16H22O4
Structural formula:



B.1.3 Physicochemical properties

Table 6. Physicochemical properties of the four phthalates

Property	Substance	Value	Reference
Physical State	DEHP	Colourless oily liquid	EU RAR, 2008a
	BBP	Liquid	EU RAR, 2008b
	DBP	Oily liquid	EU RAR, 2004
	DIBP	Colourless liquid	Annex XV dossier, 2009
Melting point	DEHP	-55°C or -50°C	EU RAR, 2008a
	BBP	<-35°C	EU RAR, 2008b
	DBP	-69°C	EU RAR, 2004
	DIBP	-37°C at 1,013	Annex XV dossier, 2009
Boiling point	DEHP	385° C at 1,013 hPa	EU RAR, 2008a
	BBP	370° C at 10.10 hPa	EU RAR, 2008b
	DBP	340° C at 1,013 hPa	EU RAR, 2004
	DIBP	320° C	Annex XV dossier, 2009
Relative density	DEHP	0.98 g/cm^3 at 20°C	EU RAR, 2008a
	BBP	1.116 g/cm^3 at 20°C	EU RAR, 2008b
	DBP	1.045 g/cm^3 at 20°C	EU RAR, 2004
	DIBP		
Vapour pressure	DEHP	0.000034 Pa at 20° C	EU RAR, 2008a
	BBP	0.00112 Pa at 20° C	EU RAR, 2008b
	DBP	9.7±3.3x10^{-3} Pa at 25°C	EU RAR, 2004
	DIBP	0.01 Pa at 20°C	Annex XV dossier, 2009
Water solubility	DEHP	3 µg/l at 20°C	EU RAR, 2008a
	BBP	2.8 mg/L at 25 to 30°C	EU RAR, 2008b
	DBP	10 mg/L at 20°C	EU RAR, 2004
	DIBP	20 mg/L at 20°C	Annex XV dossier, 2009
Partition coefficient n-octanol/water (log value)	DEHP	7.5	EU RAR, 2008a
	BBP	4.84	EU RAR, 2008b
	DBP	4.57	EU RAR, 2004
	DIBP	4.11	Annex XV dossier, 2009

B.1.4 Justification for grouping

B.1.4.1 Justification for grouping

It is well-known that humans are exposed to a mixture of chemicals such as phthalates and that exposures change over time and with age. Phthalates are a group of chemicals of which some have been associated with effects on the development of the reproductive system of male laboratory animals and endocrine disrupting effects. Few epidemiological studies on phthalates and developmental effects on the male reproductive system are available; however, widespread human phthalate exposure is evident. This report deals with four phthalates classified as reproductive toxicants, DEHP, DBP, DIBP and BBP and with similar mode of action, i.e., anti-androgenic effects. A grouping of the four phthalates is further justified by their structural and metabolical similarities. They are all ortho-phthalates with alkyl side chains, linear or branched, of length C4-C6, which are known to produce similar severe reproductive effects (including effects on reproductive organs, fertility, and development) in experimental animals, especially antiandrogenic effects (Fabjan et al., 2006) . Even though the phthalates have different potency their similar mode of action makes it reasonable to perform dose addition calculations to predict the combination effects of these chemicals.

B.1.4.2 Scientific rationale for the use of the dose addition concept in risk assessement

In Chapter E.3.5, Step 5, in the process of risk characterisation according to REACH guidance (Part E: Risk characteriation) special attention to combined exposures is addressed. Combined exposures may emerge in situations where the same person is potentially exposed to the same substance in the same setting via different routes of entry into the body or from different products containing the same substance. In special cases, where exposure occurs to several very closely related and similar acting substances, the exposure evaluation and risk characterisation should reflect this aspect. The most relevant way of reflecting this is by introducing the method of dose addition.

Dose addition is often referred to as concentration addition, simple similar action or Loewe additivity. The concept has been introduced by Loewe & Muischnek in 1926, and the model assumes that all the chemicals in the mixture act on the same biological site (receptor or target organ), by the same mechanism of action, and that they differ only in their individual potency (Backhaus et al., 2004). The additive effects are described mathematically by summing up the doses of the individual chemicals in a mixture adjusted for their differences in potencies. The effect can be kept constant when one chemical is replaced with an equal fraction of an equi-effective chemical (Kortenkamp et al., 1998). Combination effects based on dose addition can also result from chemicals at or below their respective no observed adverse effect levels (NOAELs), provided that sufficiently large numbers of chemicals sum up to a suitably high total effect dose (Kortenkamp, 2007).

The equation for dose addition (4 components) is:

$$EDx_{mixture} = \left(\frac{p_1}{EDx_1} + \frac{p_2}{EDx_2} + \frac{p_3}{EDx_3} + \frac{p_4}{EDx_4} \right)^{-1}$$

Here, EDx_1, EDx_2, EDx_3 and EDx_4 are the effect doses of four chemicals that on their own produce the same quantitative effect x as the mixture, and p_1, p_2, p_3 and p_4 are the relative proportions of the corresponding individual doses present in the total mixture dose ("fraction in mixture").

Several reports that summarize and evaluate the present knowledge about combined toxic effects of mixtures of chemicals have been published the last decade years. The report "Combined Actions and Interactions of Chemicals in Mixtures" from 2003 stated that it is not advisable to recommend rigid use of any single approach of all chemical mixtures and that existing methods are too rough (Larsen, 2003). The report "Combined Actions of Pesticides in Food" from 2002 recommended that for compounds in the mixture that share a common mechanism of action, the toxicity equivalency factor (TEF) approach should be used, if possible (Reffstrup, 2002). These two reports focused on other endpoints than endocrine disruption.

The results of two recent research projects concerning cumulative risk assessment of pesticides have been published in 2009 and 2010. The paper from the EU FP 6 research project SAFE FOODS (Promoting Food Safety through a New Integrated Risk Analysis Approach for Foods) concluded that the Relative Potency Factor (RPF) approach (equivalent to the TEF approach) seems to be an appropriate method for cumulative risk assessment of the three anti-androgenic substances included in the study, and RPF values could be estimated for several reproductive developmental endpoints in the male fetus (Müller et al., 2009).

Fig. 1 illustrates how endocrine disrupting chemicals mixed at their individual NOAELs can have effects that can be predicted by the dose addition model. In this example, three anti-androgenic chemicals have no individual effects on anogenital distance (AGD) in rats, but a statistically significant effect is seen when the three chemicals are combined at their individual NOAELs. This effect can be predicted by the dose addition model (green column). Anogenital distance is a sensitive endpoint for anti-androgenic effects in male offspring.

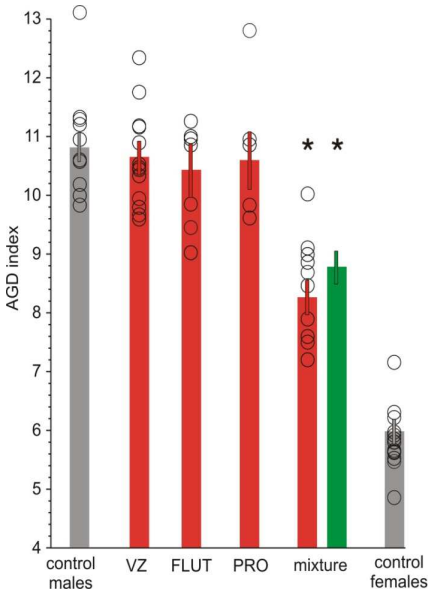


Figure 1. Mixture effects on AGD at low doses of individual mixture components, modified from Hass et al., 2007. Results shown are group mean ± 95% confidence belt for control males and females (gray), individual doses of 24.5 mg/kg vinclozolin (VZ), 0.77 mg/kg flutamide (FLUT), and 14.1 mg/kg procymidone (PRO) (red), the combined mixture dose of 39.37 mg/kg (red), and the predicted mixture effect (green) based on dose addition. Open circles represent litter means. *$p < 0.05$ compared to control.

Other examples in the literature also show that the effects of combinations of endocrine disrupting chemicals belonging to a similar class do not deviate substantially from dose addition. These results are a good indication that dose addition can be adopted as the default concept for risk assessment of endocrine disrupting chemical mixtures (Howdeshell et al., 2008 and 2007, Christiansen et. al., 2009, Rider et al., 2009 and Kortenkamp & Faust, 2010).

When considering the currently available scientific evidence as well as pragmatic considerations the idea of adopting dose addition as the preliminary default concept for cumulative risk assessment and prediction of mixture effects is supported. As described below this is carried out by current practice in many regulatory bodies in e.g. the EU, USA and by recommendations of international bodies (Kortenkamp et al., 2009).

In June 2009 a report from an Expert workshop on combination effects of chemicals was published. The workshop was organized under the auspices of the Danish Ministry of the Environment and the Danish Environmental Protection Agency and gathered international experts to discuss endocrine disrupters from a regulatory perspective. The application of dose addition as an assessment method was recommended as a default, until evidence as to the suitability of alternative assessment concepts emerges. It should replace the current risk assessment paradigm that is focused on single chemicals, without considering contribution from other substances (Kortenkamp & Hass, 2009).

Dose addition models can in most cases predict the combined effects of anti-androgens and demonstrate that marked effects can occur at mixture doses below the NOAELs for the single chemicals (Christiansen, 2009; Christiansen et al., 2009).

In relation to phthalates, an important recent example is the establishment of a US National Academy of Sciences committee on cumulative risk assessment for phthalates and related chemicals at the request of the US EPA. A report of this committee was made publicly available in December 2008 and in the summary of this report is stated: "*Thus, the evidence supports the use of dose-addition as an approximation in estimating cumulative risk posed by phthalates and other anti-androgens. The use of a dose-addition model is also supported by data that show cumulative effects at doses at which individual mixture components did not induce observable effects*" and moreover; "*Cumulative risk assessment based on common adverse outcomes is a feasible and physiologically relevant approach to the evaluation of the multiplicity of human exposures*" (NCR, 2008).

In December 2009 a report called "state of the art on mixtures" was published. This report details the findings of a project on mixture toxicology and ecotoxicology commissioned by the European Commission. It describes the scientific state of the art in the field, and gives an account of the regulatory state of the art for dealing with combined exposures in EU, USA, Japan and in international bodies. Here it was stated that there is a consensus in the field of mixture toxicology that the customary chemical-by chemical approach to risk assessment might be too simplistic. It is in danger of underestimating the risk of chemicals to human health and to the environment. Moreover, it was concluded that here is unanimous agreement across all disciplines that, in the case

of mixtures of similar compounds, combination effects require special consideration (Kortenkamp et al., 2009).

Based on both scientific literature and detailed reports (NCR, 2008) on this subject the results indicate that the mixture effects of phthalates are proved adequately predicted with dose addition models, even though a variety of mechanisms of action clearly are involved.

The use of a dose-addition model is also supported by data that show cumulative effects at doses at which individual mixture components did not induce observable effects. A report from Danish EPA on chemical exposure of 2-year old children is also a good example that supports the recommendation of dose addition (Danish EPA, 2009). That report was based on an analysis of the exposure of 2-year olds to potentially endocrine disrupting chemicals in their surroundings, and the report evaluated whether the daily cumulative exposure to these chemicals present a risk to the developing child (Danish EPA, 2009).

Because of the similar mode of action of the four phthalates in question, the concept of dose addition is used to calculate the cumulative risk of the four phthalates. The differences in potencies are delt with as individual DNEL values have been set for the four phthalates (see section B 5.9 Toxicity for reproduction). The four phthalates also have differences in toxicokinetic which is addressed by using different absorption rates depending on the phthalate and the route of exposure (see section B.5.1).

B.2 Manufacture and uses

B.2.1 Manufacture, import and export of a substance

As can be seen in section Table 7 articles for use indoor and/or direct human skin contact in total contain 265,000 tonnes per year of the four phthalates, DEHP, BBP, DBP and DIBP. DEHP represents 95 percent of this. The imported articles represent at least 40,000 tonnes (16 percent).

Table 7 Estimated tonnage in end-products marketed in the EU in 2007 (ECHA, 2009a-d).

Articles	Tonnage, t/y					% of proposed restricted use	% of total use
	Four phthalates	EU-Manufacture of articles	Import	Export	End product use		
Flooring	DEHP	33,000	2,000	4,800	30,200	12.8	10.6
	BBP	4,290			4,290	58.3	53.6
	Total	37,300			34,500	13.4	11.5
Wall covering	DEHP	11,000	700	1,600	10,100	4.2	3.5
Film/sheet, coated and moulded products	DEHP	97,400	18,500	18,500	97,400	39.7	34.2
	BBP	900			900	12.4	11.4
	Total	98,300			98,300	38.1	32.6
Wires and cables***	DEHP	52,000	6,200	5,600	52,600	22.4	18.5
Hoses and profiles	DEHP	31,000	1,600	3,000	29,600	12.6	10.4
Other Polymer applications	DEHP	12,300	10,900	3,100	20,100	8.6	7.1
	DBP	2,930			2,930	55.1	35.5
	Total	15,830			23,030	8.9	7.6
Adhesives, sealants and grouting agents**	DEHP	4,000	n.d	n.d	4,000	1.7	1.4
	BBP	1,920			1,920	26.1	24.3
	DBP	1,980			1,980	37.2	24.0
	Total	7,900			7,900	3.1	2.6
Lacquers and paints**	DEHP	500	n.d	n.d	500	0.2	0.2
	BBP	160			160	2.2	2.0
	DBP	160			160	3.0	1.9
	Total	820			820	0.3	0.3
Printing ink**	DEHP	1,000	n.d.	n.d	1,000	0.5	0.4
Other non-polymeric	DEHP	20	n.d.	n.d	20	0.0	0.0
	BBP	80			80	1.1	1.0
	DBP	250			250	4.7	3.0
	Total	350			350	0.1	0.1
All articles intended for indoor use and/or with human skin contact.	DEHP	242,200	39,900	36,600	245,500		86.1
	BBP	7,360	n.d.	n.d.	7,360		92.0
	DBP	5,230			5,320		64.5
	DIBP*)	6,930			6,930		
	Total	261,800			265,100		85.7
All articles on EU market	DEHP	282,200	39,900	36,600	285,500		
	BBP	8,000	n.d.	n.d.	8,000		
	DBP	8,250			8,250		
	DIBP*)	10,750			10,750		
	Total	309,200			312,500		

* Calculated on the basis of information in ECHA 2010b and 2009b. DIBP has not been distributed to the types of articles

** Products that are mixtures and therefore not covered by the restriction proposal unless part of an article

*** Cables included in this table are cables not above 1000 kW. Some cables below that capacity are for use out doors.

There is no specific information on the content of DIBP in articles. However ECHA (2010b) mentions that in an authorised IUCLID data sheet from 2000 the quantity of DIBP manufactured and/or used in Europe is indicated in the range of 10,000 to 50,000 t/y. DIBP has been reported in the literature as one of the alternatives employed for DBP. The volume for DIBP given in table 7 is based on information on export of DBP and DIDP given in ECHA 2009b and information given in registration dossiers mentioned below.

A screening of the registration dossiers submitted by producers and importers in 2010 indicates that the overall production volume of the four phthalates in 2009 or 2010 is app. 230,000 tons, of which

210,000 tonnes is DEHP. The export data was not collected. Due to confidentiality issues the figures for the other 3 phthalates are aggregated. Of the 20,000 tonnes produced or imported about 7,000 tonnes was exported. DIBP constitutes the largest part of the three phthalates used in the EU.

As shown in table 8, the total production and import of the four phthalates in 2007 in EU is estimated to be 401,000 tonnes, of which 309,000 (77%) was for the EU market. Assuming the same proportion of the produced and imported DEHP in 2009 as for 2007 and information in registration dossiers on the three other phthalates the total use of the four phthalates in EU is estimated to 184,000 tonnes.

Table 8. Estimated production of the four produced phthalates in end-products marketed in the EU in 2007

	2007			2009-10	
	EU production + import of subtances Tonnes	Export of substances and mixtures Tonnes	EU production and import used for production of articles Tonnes	EU production + import of substances Tonnes	EU production and import used for production of articles Tonnes
DEHP	347,000	65,000	282,000	210,000	171,000[1)]
BBP	20,000	12,000	8,000	20,000	13,000
DBP	10,000	15,000	19,000		
DIBP	24,000[2)]				
Total	401,000	92,000	309,000	230,000	184,000

Source: (ECHA, 2009a-d) and registrations dossiers submitted in 2010.

1) Calculated assuming the same relation (65/347) between export and production/import as in 2007
2) Estimated on the basis of export (ECHA 2009b and information in IUCLID dataset (ECHA 2010b)

Hence it can be seen that the use of the four phthalates in 2009-10 seems to be 60 % of the use in 2007 (From 309,000 tonnes to 184,000 tonnes). The reduction is remarkable as the use of DEHP in 2007 was very close to the use in 2005 and 2006.

Furthermore assuming an equal substitution rate between EU produced articles for use indoor and for contact with human skin and other EU produced articles the content for the four phthalates in the first mentioned group is 157,100 tonnes (0.60*261,800).

There is no new data available on the content of the four phthalates in imported articles. However it is not likely that the substitution rate has been as big as in the EU, where much focus has been on the four phthalates in relation to the inclusion in Annex XIV to REACH. Assuming a proportional reduction in the content of the four phthalates in exported articles and a content in imported articles of 35,000 tons the total content in the relevant articles (both imported and EU produced) is estimated to be 170,000 tonnes, implying a reduction of 36 % from 2007.

Data on plasticisers used in imported products are scarce or lacking for products where production in the EU dominates (e.g. flooring of vinyl), as with a few exemptions it has not been possible to identify the companies responsible for the import (see sections below).

The data available has thus not enabled a full quantification of the four phthalates and other plasticisers present in imported articles and EU production of the articles covered. Varying information regarding quantities and concentrations is available for different plasticisers in the different fields of application, see section B.2.2.

The trends in the market concerning use of the four phthalates and use of alternatives are described in section B.2.2 under the different main article groups and in section E.

B.2.2 Uses

The main use (94 %) of the four phthalates contained in articles proposed for restriction is in PVC. Minor uses are in non-PVC polymers and non-polymers.

Three of the four phthalates (DEHP, DBP and BBP) are regulated in toys for children and childcare articles. Several other articles apart from children toys and childcare articles may cause exposure of especially children but also adults to the four phthalates used as plasticisers in these articles.

The following article groups were selected for getting information on the use of the 4 phthalates, use of alternatives and the financial implications for the use of alternatives

- Flooring (and heavy wall covering)
- Insulation on wires and cables
- Electronic devices
- Plast coated fabric and film/sheets used for bags and brief/suitcases and similar items
- Plast coated fabrics and film/sheets used for tablecloth, curtains, shower curtains and similar items (not industrial uses)
- Carpet tiles/squares produced with (typically) PVC-foam as back cover
- Water- and air mattresses
- Plast coated wallpaper/tapestry
- Footwear
- Bathing equipment (swim-coats/wings/belts and pools - inflatable and others)
- Balls for training and physical exercises
- Others: Erasing rubber

The reasons for choosing these article groups for this type of study were several. It was those groups of articles where possible exposure were expected and - for some of the groups - where it was known that substitution had already taken place for articles within the group. Information form such article groups could give useful information on alternatives and related financial implications. It should also be mentioned that these groups were generally the groups that the Danish EPA knew of in this respect at the onset of the preparation of the dossier that contributed to the exposure in respect to the targeted effect and that no information has been received during consultations with industry about other uses.

The dossier submitter had hoped for information on uses in other articles /article groups during the work with the dossier, e.g. through the many contacts with industry and importers. This was however not the case, and the expected information in the registration dossiers – available in spring 2011 - on uses in individual articles was not given in (any) sufficient detail.

One obvious challenge in getting information is the lack of knowledge for imported articles that only enters the European market on a “day-to-day” basis, typically different cheap articles made of PVC sold by “direct sale” retailers. These articles could be any kind of articles but they are impossible to define either as a group or individually.

However, the treated article groups constitute the vast majority of the known amounts used for articles within the EU. The figures are however a bit difficult estimate due to the changes in the import/production in the registration dossiers compared to the estimated amounts given in the ECHA reports from 2009 (Echa 2009 a-d) on “Data on manufacture, import, export, uses and releases of the 4 different phthalates” and the figures calculated in the report on the individual article groups on which the data presented in B.2.2.1-B.2.2.12 are based (Danish EPA 2011).

The article groups discussed below in this section cover a broad range of different articles. As mentioned before, the dossier submitter has no information on other actual uses, but as the groups are so widely dispersed and covering the broad range other unknown remaining uses could be expected to be within the range of the already treated article groups.

To obtain reliable figures on the use and quantity of plasticisers in the import and EU production of selected fields of application has been a complicated task (for both the four phthalates and the alternatives) i.a. due to the number of companies involved in such activities in EU. The strategy has been to combine statistical data of EU production and import to EU of the application areas in question with expert assessments and estimates obtained from European trade organisation and European companies on the types and quantities of plasticisers being utilized (see chapter G for a list of contacted companies/organisations).

It has been very difficult to obtain the needed detailed data from industry with only a few exemptions. Therefore, besides trying to get information from stakeholders throughout Europe, an alternative approach has been to develop the required information concerning the market and product groups based on contact to individual Danish and international companies. This approach has been adopted for the majority of the applications areas investigated. The approach, however, have some weaknesses as discussed more in detail in section E.4.

Below is the information available for each of the afore mentioned fields of application presented. The bulk of information is coming from a report the Danish EPA conducted via a consultant in 2010 (Danish EPA, 2011) unless otherwise stated (main difference is surveys made by the Danish EPA in 2010, Danish EPA, 2010a-c). The references mentioned can be found via that report. The information given below is a very short extract of the information given in the report, and in order to get more detailed information (as far as this has been possible to obtain) please refer to the report.

B.2.2.1 Flooring of vinyl and heavy style wall covering

Definition of the product group
Flooring and heavy style wall covering made of PVC are vinyl materials with and without textile or PUR backing material. The thickness of the material will generally be in the range of 1-3 mm. The products may be used for covering of ceilings as well.

Several different qualities of flooring are marketed for different customer segments according to contacted vinyl flooring producers. So-called heterogeneous and homogenous types are mainly marketed to professional users requiring high wear resistance. For the private market, mainly the softer type "cushioned vinyl" is marketed. Cushioned vinyl consists of plasticised foamed PVC with an integral smooth surface (skin), or of a plasticised PVC film upper, backed by foamed PUR. Cushioned vinyl is most likely the quality which is also sometimes used for wall covering in bathrooms, etc.

Import to and production of articles in EU
Import of wall covering and flooring materials to EU27 is estimated to around 112,000 tonnes pr. year and manufacturing within the EU is estimated to around 1,400,000 tonnes pr. year. The dominance of EU production was confirmed via contact to producers in the EU. Import from the Far East was, however, also mentioned.

Plasticisers in use
The total consumption of DEHP for flooring vinyl products has been estimated at 30,200 tonnes per year in 2007, based on updated DEHP production statistics in combination with partly older consumption distribution estimates and import/export statistics. EU-manufacture dominated this application of DEHP. The total consumption of EU-produced BBP for flooring products was estimated at 4,290 tonnes per year for 2007. Here, import/export were however not included in the estimation process. Flooring was considered the dominant application of BBP, as about half of the BBP produced in the EU was used for this application ((ECHA, 2009b). The similar ECHA report (ECHA, 2009c)) on DBP mentions flooring as one of the product types where DBP may be used, but does not provide specific estimates of the consumption for this application.

Based on the import and production statistics and the plasticiser concentrations mentioned above, the total plasticiser consumption for EU produced flooring is expected to be within the range of 140,000 - 430,000 tonnes per year. Based on the mentioned statements from manufacturers, most of this is presumably DINP. Similarly, the plasticiser consumption in imports is expected to be in the range of 11,000 - 34,000 tonnes per year. In imports, the frequency of DEHP usage may be larger, as DEHP is still the dominant general plasticiser globally (50% of total phthalates consumption in 2007 across all applications according to ECPI (ECPI, 2010)).

The European industry association on these products is the European Resilient Flooring Manufacturers Institute, ERFMI. ERFMI has provided data on the content of DEHP, BBP, DBP and DIBP in average vinyl flooring products in the European market around 2005. The data are shown in Table 9. Total plasticiser concentrations or concentrations of other plasticisers could not be provided. As total plasticiser concentrations generally are in the range of 10-30%, a plausible explanation may be that other plasticisers are used in the production of flooring. ERFMI informed that they were not in a position to provide other data requested in the questionnaire, and forwarded the questionnaire to their members (with no resulting response) (ERFMI, 2010b)).

Table 9 Concentrations of selected phthalates in average flooring products (2005 data) ((ERFMI, 2010a)

	DEHP %	BBP %	DIBP %	Other unspecified phthalates
Homogeneous PVC	0.57			
Heterogeneous PVC	3.0	0.89	1.59	
PVC with foam Backing		0.44	0.65	
Laminated PVC	None	None	None	None
Cushioned PVC	1.36	0.64	5.71	
Safety PVC				1.40
Semi Flexible PVC	*1.54*			

The Danish Trade organisation for flooring "Gulvbranchen" has earlier informed that since 2000 only DINP and DIDP are used in PVC flooring and wall covering marketed in Denmark (Christensen et al 2007).

The Danish EPA has investigated the content of phthalates in PVC floorings and wall coverings in both 2001 and in 2010 (Danish EPA, 2001 and Danish EPA 2010a). In 2001 five different PVC floorings were analysed for the content of phthalates showing that four of the five floorings contained at least one of the phthalates DEHP, DBP or BBP in concentrations above 1 % and the last flooring contained a mix of DINP and DIDP.

8 PVC floorings were analysed quantitatively for the content of DEHP, DBP, DIBP and BBP in 2010. These analyses showed that only one of the analysed floorings had a content of one these four phthalates in a concentration above 1 % (DIBP, 7.4 %). In four other PVC floorings one or more of the four phthalates were found in individual concentrations below 0.1 %. Six of the floorings had a content of DINP, but the content was not quantified (Danish EPA, 2010a).

German investigations performed in 2003 ((Stiftung Warentest, 2003) revealed a rather complex picture regarding plasticiser usage. PVC flooring marketed in Germany contained all the following phthalates: DIBP, DBP, BBP, DEHP, DINP, DIDP, DIHP and DIOP. DINP and DIDP were found in significant concentrations. A total of 25 different products were analysed. The total concentration of phthalates registered in the products was in the range of approx. 6.3% to 36.5%. The content of the individual phthalates was registered as follows: DIBP: ≤6.9%, DBP: 1.3%, BBP: ≤6.8%, DEHP: ≤13.6%, DIHP: ≤33.0%, DIOP: ≤1.1%, DINP: ≤22.0% and DIDP: ≤1.9% ((Stiftung Warentest, 2003). Most products contained a mixture of different phthalates.

Confidential industry information has confirmed a possible continued use of DBP in PVC flooring products.

These German results are in line with the consumption data from the ECHA reports on DEHP, BBP and DBP ((ECHA, 2009a, ECHA, 2009b, ECHA, 2009c) The discrepancy between these data and the information provided by major European producers during 2010 may be caused by a continued trend away from DEHP, BBP and DBP over the last few years, or this trend may be most clearly expressed for some major European producers, whereas other EU producers and imports may not have changed their plasticiser usage similarly.

Alternatives available
DINP, DIDP, DIHP, DIOP, castor oil derivatives (COMGHA), dibenzoates like DGD (a secondary plasticiser (Maag et al., 2009) are used in PVC flooring products.

According to a producer, the usage of the plant oil derivative COMGHA did not cause any changes in the product quality, as this alternative plasticiser performed similarly to the phthalates otherwise used.

Trends and perspectives
For the EU production of flooring and wall covering vinyl, the movement away from DEHP, BBP, DBP and DIBP is expected to continue. The case for imports is unknown except that alternative plasticisers are now also used for example in China for various other product types.

B.2.2.2 Insulation on wires and cables

Definition of the article group
The article group consists of isolated electrical wires and cables, as well as optical fibercables, of types used indoor in homes and offices. PVC is in reality used as insulation as well as coating material. Cable and wire types used in the homes include flexible cables used for connecting electrical devises, construction cables for low voltages, 230 and 400V, low voltage cables used inside electrical and electronic devices, and optical cables.

Import to and production of articles in EU
Import of cables to EU27 is estimated to around 500,000 tonnes pr. year, while production in EU27 is estimated to around 2,900,000 tonnes pr. year – both import and production spreads over several types of wires and cables.

Plasticisers in use
Based on detailed data from a cable catalogue of a European producer, the average PVC content of regular PVC insulated wires and cables is around 30% for single solid copper conductor wire (used for 230-400V installations), whereas it is around 65% for 2-3 conductors flexible connecting cords used in the home or office, and around 70% for 3- and 5-conductors construction cables (230-400V installations).

Based on information from cable manufacturers DIDP, DINP and DEHP are likely the main plasticisers used for cables in the EU. According to one manufacturer, DIDP constitutes about 80% of the current plasticiser consumption for cables in the EU.

BBP, DBP and DIBP are not reported used in cable and wire, probably due to their high volatility. Cables are heated during use and this increases the volatilisation. However, confidential industry information has indicated a possible continued use of DBP as secondary plasticiser in wire and cable.

The total consumption of DEHP for cable and wire used indoors has been estimated at 52,600 tonnes per year in 2007 (ECHA, 2009a).

Alternatives available
PVC insulation is plasticised with DIDP, DINP, DPHP, TOTM and adipates. PVC-free cables are available with insulation made of PE or silicon rubber.

According to contacted producers there are no uses of indoor cable types for which DEHP cannot be substituted.

Trends and perspectives
Manufacturers stated that there is a continued trend away from DEHP (BBP, DBP and DIBP are not used – see above regarding confidential information about DBP). The case for imports is unknown.

B.2.2.3 Electric and electronic equipment (EEE)

Generally the use of DEHP in electrical and electronic equipment (EEE) is not deemed essential as technically suitable alternatives are available and already used extensively today; especially DINP is an easy-to-used alternative. The use of DBP and BBP in EEE is not deemed essential as technically suitable alternatives are available and already used today for similar applications as the possible applications in EEE, however for some specific non-polymer applications substitution may be particular difficult. European Plastic Converters (EuPC), has in a survey by their members not indentified any use of DBP, and assume that DBP today is used by relatively few companies for different niche purposes. All available data indicate that alternatives exist, for example DGD, Benzoflex 2088 and ASE. For PVC softening, omitting the use of these secondary plasticisers may also be technically possible, although probably with increased PVC processing expenses as a consequence (Danish EPA, 2010d).

The use of secondary plasticisers, for example to improve plasticising performance and permanence at elevated temperatures as in electrical cables, is generally applied already, and a change of primary plasticiser is not expected to have major consequences as regards special performance requirements. Normal re-adjustment of the formulation of the system consisting of the polymer, primary plasticiser, secondary plasticisers and other additives will however likely be needed in most cases. It cannot be ruled out completely that some niche productions for specialised purposes in some EEE may have difficulties in substituting DEHP, but no evidence of such niche production has been encountered (Danish EPA, 2010d).

B.2.2.4 Bags, brief-/suitcases and similar items

Definition of the product group
The product group covers plasticised (mainly PVC) parts on bags, brief- and suitcases and similar items. The PVC parts in questions includes thin PVC film typically used inside the bags and cases, PVC coated fabrics and leather-look, and PVC marks, figures, profiles sewn, welded or otherwise attached to the outer surface or bottom of the bags and cases.

Import to and production of articless in EU
Import of bags to EU27 is estimated to around 690,000 tonnes pr. year, while production in EU27 is estimated to around 937,000 tonnes pr. year – both import and production spreads over several types of different items.

<u>Bags in general</u>
There are numerous bag producers with each their choice of design. It has not been possible to identify a European trade organisation able to provide an overview of the main trends in the market. Thus the choice has been made to focus on the Danish market and use data on this market as

representative for the European market. The overall picture is however motley and it is not possible to give a clear view of the main stream of the bag market.

A dominant vendor of bags in Denmark informs that approx. 20% of their bags contain PVC. According to this vendor the content of PVC in bags varies from bags made entirely or almost entirely of PVC to bags containing only small fractions of PVC, as PVC in these cases is used mainly for decorations and similar purposes. According to the vendor, the choice of materials is the same all over EU.

Assuming that 20% of all bags contain PVC, and that 70-90% of these bags contains in the range of 1-5% PVC, while the remainder is made entirely of PVC, the quantity of PVC in bags imported to EU is roughly estimated to 15,000 - 46,000 tonnes. Production in EU may similarly be estimated to 20,000 - 63,000 tonnes PVC. It is emphasized that these estimates should be taken as an indication of the probable order of magnitude only.

School bags

It is estimated by producers, that approx. 100,000 - 110,000 school bags is sold per year in Denmark. No precise data on the number of school bags sold in EU is available. Assuming a population of EU27 of approx. 500 mio. inhabitants and a population of Denmark of approx. 5.5 mio. inhabitants (Eurostat 2010) the total number of school bags sold in EU may roughly be estimated to 9-10 mio. bags yearly.

Plasticisers in use

Bags in general

It has not been possible to obtain information from manufacturers about the specific plasticisers used, due to confidentiality. The actual producers of the PVC bags are also regarded as confidential, leaving no ways to obtain further information about the production.

The Danish EPA has investigated the content of phthalates in bags in both 2001, 2007 and in 2010 (Danish EPA, 2001; Danish EPA, 2007 and Danish EPA 2010b). The bags investigated in 2010 are bags for children and the results from these analyses are therefore reported in the section below regarding school bags and similar. In 2001 three bags were analysed for the content of phthalates. The results of the analyses showed that all three bags contained DEHP, in concentrations from 12 to 21 %. One of the bags also contained a mix of DINP and DIDP at 11 % and a low concentration of BBP, below 1 %.

School bags and similar

A dominant Danish importer of bags for children informs that their bags are manufactured in China and does not contain phthalates. No information on the actual plasticisers being used has been available.

The same picture has been found in Germany. A test of 12 school bags revealed that all bags were free of phthalates (Stiftung Warentest, 2009). The content of PVC in the tested bags is not known as this information has not been registered.

In a survey from the Danish EPA from 2007 school bags and toy bags were analysed for the migration of certain substances to artificial sweat. This showed that six of the seven analysed bags migrated DEHP, DIBP or DBP to artificial sweat, indicating that these phthalates are used as plasticisers in part of the bags.

The survey from the Danish EPA from 2010b, 10 plastic bags for children were analysed for the content of DEHP, DBP, DIBP and BBP. The bags were limited to handbags, shoulder bags, sponge bags, rucksacks, trolleys and plastic bags for consoles (such as Nintendo DS), and the analysed bags did not include school bags. The results showed that four bags contained DEHP in concentrations above 1 %, the concentrations of DBP and DIBP were below 0.1 % in all of the bags and BBP was not detected in any of the bags. One of the bags contained DINP, but the content was not quantified.

It has not been possible to find evidence for the use of phthalates in school bags in Denmark. Whether or not this reflects the actual situation in the rest of the EU is unknown. However, the use of DEHP in other types of bags for children has been detected.

Available altenatives
Producers and suppliers to the Danish market have informed that alternatives are available for all parts of bags.

A Danish producer informs that PVC in reflexes on bags has been substituted with the polymer TPX (polymethylpentene**)** within the latest 2-3 years.

A Danish importer of children's bags made in China informs that they are guarantied by their supplier, that phthalates are not used. Apart from that PVC is still the material used, no information has been provided about the choice of plasticisers adopted.

The survey from the Danish EPA (2010b) also shows that it is possible to produce plastic bags without a content of the four phthalates.

Trends and perspectives
According to a dominant vendor on the Danish market the trend for bags in general is that no changes occur without regulation. Therefore, the PVC and phthalates will not be substituted unless rigorous legislation is implemented.

Regarding school bags, the picture is slightly different as the general concern related to PVC and phthalates has already motivated the dominant Danish manufacturers to develop PVC free products. As mentioned above, in other bags intended for children some were found to contain DEHP in concentrations > 1 %. The trend in other EU countries is not known.

It is not known to what extend minor manufacturers (or importers) of school bags and similar still using the four phthalates has planned to adopt alternatives in the near future.

B.2.2.5 Tablecloth, curtains, shower curtains and similar items -not industrial use

Definition of the product group
The product group covers tablecloth, curtains, shower curtains and similar items made of PVC film or coated fabrics for home and office purposes but not for industrial purposes.

Import to and production of articles in EU
Import of tablecloth, curtains, shower curtains etc. to EU27 is estimated to be around 84,000 tonnes pr. year, while production in EU27 is estimated to be around 1,500,000 tonnes pr. year. It has to be noticed that there are very big uncertainties regarding this estimation due to the very diverse article group. Besides this, the statistical positions used in the estimation may contain significant amounts

of items used for other purposes than tablecloth, curtains, shower curtains, etc. Such other items could include for example PVC film and sheet used for industrial purposes.

Plasticisers in use
The EuPC (2010) has reported that the use of DEHP may be more widespread than the average 15% (or so) DEHP share in plasticiser use in Europe for the application "flexible PVC film products". EuPC estimated that about 50% of such products would contain DEHP, whilst the actual use would vary from company to company. The applications which reported a high use were stationary (office supply), tapes, shower curtains and then those applications where technical or legal requirements impose the use of DEHP such as medical applications.

Several producers of flexible PVC film for these purposes have been contacted, but only one company has responded positively. According to this company, the use of DEHP, DBP and BBP is widespread for these applications. The company does however not use them themselves, and front-end producers, mainly in the EU, are now working at reducing and/or substituting these substances with others non-SVHC plasticisers. The company informs that PVC table cloth with textile backing (often non-woven textiles) contains around 90% plasticised PVC and 10% backing material.

The Danish EPA has analysed the content of phthalates in three shower curtains in 2001. The analyses show that all three shower curtains contain DEHP in concentrations between 6.7 and 22 %, and that one of the curtains also contained DINP and DIDP, the total concentration was 8.6 % (Danish EPA, 2001). Besides this, the Danish EPA made analyses of DEHP, DBP, DIBP and BBP in tablecloths and dinner mats, shower curtains, and child appealing shower curtains and curtains (Danish EPA, 2010a & Danish EPA, 2010b).

12 oilcloths and dinner mats have been analysed. The analyses showed that 4 oilcloths had a content of DEHP above 1 % (up to 25%), the concentration of DBP and DIBP were below 0.1 % in all of the analysed products and BBP was not detected in any of the oilchlths and dinner mats. DINP was detected in four of the oilcloth and dinner mats, but the content was not quantified (Danish EPA, 2010b).

The Danish EPA analysed 20 shower curtains in 2010 (Danish EPA, 2010a & Danish EPA, 2010b). 10 of the shower curtains were child appealing. Five of the child appealing shower curtains contained DEHP in concentrations above 1 % (up to 30%) and 6 of the other shower curtains contained DEHP in concentrations above 1 % (up to 28%). Seven of the child appealing shower curtains contained DINP, but the content was not quantified. DINP was not qualified in the other shower curtains.

A Danish survey of selected non-toy products which could cause exposure to children included a PVC shower curtain containing 26% DEHP, a dish mat with 11% DEHP, and a transparent table cloth (PVC film) with 14% DEHP and 3,2% DINP (IMS, 2009).

Alternatives available
As mentioned above DINP is used as substitutes for DEHP in table cloths, dinner mats and shower curtains.

Other plasticisers than phthalates in use for tablecloth/cover are ATBC, DINCH and DOA in combination with ESBO.

Based on a limited internet search, various alternatives to PVC shower curtains are available at low costs. Many market synthetic, woven textiles, for example of polyester, but also plastic film curtains of EVA/PEVA are marketed (IKEA 2010).

European retailers are marketing PVC-free plastic coated table cloths (oil cloth style), for example coated with acrylics.

European producers inform that phthalates-free table cloth/covers of PVC film and PVC-coated textile are available on the European market. Plasticisers used include, among others, "TBC (tributyl citrate)" (probably actually ATBC, often used for PVC for food contact), DINCH, DOA and ESBO.

Trends and perspectives
No general trend has been identified for EU-production. The case for imports is unknown except that alternative plasticisers are now also used for example in China for various other product types.

B.2.2.6 Carpet tiles/squares with PVC-foam as backing

Definition of the product group
The product group covers carpet tiles and squares produced with PVC, foamed or not, as back cover. The market is today dominated by carpet tiles with other backing materials than PVC.

Import to and production of articles in EU
Import of carpet tiles to EU27 is estimated to be around 6,000 tonnes pr. year, while production in EU27 is estimated to be around 1,425,000 tonnes pr. year. No data on manufacturing of carpet tiles in EU27 are available. The figures available include carpets and thus clearly overestimate the quantity of carpet tiles manufactured.

The EU professional market for carpet tiles is estimated at some 25-40 million m^2 per year. According to a major producer, the typical weight of carpet tiles is around 4 kg/m^2, meaning that this equals some 0.1-0.16 million tonnes per year. Compared to the figures presented, this means that import of carpet tiles into the EU is minimal.

Plasticisers in use
Based on the survey in this study, most carpet tiles marketed in the EU is made with other backing materials than PVC. Of five contacted European producers of carpet tiles, three used other backing materials than PVC (bitumen, textiles), and two had production of carpet tiles with PVC backing. The contacted producers believed that the total market share for PVC-backed carpet tiles to be low. Based on their information the market share is estimated here to be around 10-15% of the total carpet tile market.

Information from producers of carpet tiles with PVC backing indicate that today, only DINP is used as plasticiser in the PVC backing of carpet tiles in the EU.

The Danish EPA has analysed 8 carpet tiles for the content of DEHP, DBP, DIBP and BBP. None of the analysed tiles contained these four phthalates in concentrations above 0.1 %. Only in one of the carpet tiles DIBP was detected above the detection limit, but in low concentrations (0.016 %) (Danish EPA, 2010a). These results confirm the information from the producers.

Alternatives available

Alternative backing materials covers bitumen and textiles. DINP is used in PVC backing. DOTP, DIHP and DINCH are mentioned by producers as alternatives to DEHP, and isodecyl benzoate is mentioned as an alternative to BBP.

Adipates, phosphates and trimellitates are also mentioned as potential alternatives, yet one producer states that some of these may not be suitable technically.

Trends and perspectives
For EU production substitution of the four phthalates seems to be completed already. A producer reported that BBP was substituted (or abandoned) in the late 1980's and DEHP was substituted around 2000.

The case for imports is unknown.

B.2.2.7 Water beds and air mattresses

Definition of the product group
The product group covers water beds and air mattresses produced of PVC film or coated fabrics.

Import to and production of articles in EU
Import of air mattresses to EU27 is estimated to be around 10,500 tonnes pr. year, while production in EU27 is estimated to be around 1,200 tonnes pr. year

10,516 tons (27.2 million €) of pneumatic matressess of textile format are imported into the EU and the EU production of these product is 1,167 tonnes (6.5 million €). The figures on manufacturing also include other camping items and cannot be compared directly with import figures. No data on import and manufacturing of water beds in EU27 are available.

It should be noted, however, that the import of air mattresses seems to be large compared to the quantity manufactured in EU27.

Plasticisers in use
Water beds
Water bed mattresses are made of flexible PVC with different barriers of PVC or other materials inside the mattress. In addition, the water beds generally contain a PVC safety liner which can contain the total water amount in case of leaks. Of the contacted major European producers of water beds, 2 out of 4 producers use only Mesamoll II (ASE, alkyl sulfonic acid ester of phenol). One producer has two product ranges, one with Mesamoll II and one with unspecified phthalates. One producer used unspecified non-phthalate plasticiser(s). One producer estimates that some 40-50% of the world market for water beds is produced with Mesamoll II today, while another producer estimates that only 10-20% of the marketed water beds in the EU do not contain phthalates.

Extrapolation from sales in Germany and Austria to the whole EU population indicates a sale of perhaps 100.000 - 200,000 water beds annually. This figure has been evaluated as a "reasonable estimate" by one of the major producers. Plasticiser concentrations in PVC in water beds are assumed to similar to the film used in air mattresses, namely 20-30%. With a flexible PVC amount of around 25-30 kg per water bed according to a major producer, this corresponds to some 3,000-6,000 tonnes PVC and 600-1,800 tonnes plasticiser.

Air mattresses

PVC air mattresses now dominate the market according to a producer, while traditional rubber/cotton air mattresses are also available, but may be loosing market. Of the 3 major producers of airbeds contacted (all with actual production outside the EU) one company uses DINP in their air mattresses, while another uses so-called "non-3P" film, meaning PVC film without DEHP, DBP and BBP, but with unspecified plasticisers. The third company did not wish to provide information on their plasticiser use due to the competition in a price-sensitive market. According to one producer, large parts of the world production of air mattresses today use "non-3P" PVC film.

The Danish EPA has analysed 13 air mattresses for the content of DEHP, DBP, DIBP and BBP (Danish EPA, 2010a). Four of the analysed mattresses had a concentration of DEHP above 1 % varying from 8.2 to 30.4 %. DIBP was detected in one of the mattresses in concentrations below 0.1 % and DBP and BBP were not detected in any of the analysed mattresses. Eight of the mattresses contained DINP, but the content was not quantified. Two of the mattresses containing DEHP in concentration above 1 % also contained DINP (Danish EPA, 2010a).

Assuming that the plasticiser concentration generally is between 20 and 30 %, as reported by one producer, and confirmed by the analyses from the Danish EPA (Danish EPA, 2010a), that the EU manufacturing of "pneumatic mattresses and other camping goods (excl. tents, sleeping bags etc.)" is dominated by air mattresses, and that the export is marginal, the total marketed amount of some 11,700 tonnes of air mattresses in the EU corresponds to around 2,000-3,500 tonnes plasticiser annually. It cannot be ruled out that part of this may be DEHP, but available data do not allow a quantification of this possible use. If DBP and BBP are present in these products at all (not verified), it may likely be in small amounts only.

Alternatives available

ASE seems to be a major alternative for PVC film in water beds. For air mattresses DINP is mentioned by one major producer. Other producers have not specified which plasticisers they use, except that one specifies "non-3P" usage.

For air mattresses - as stated above - the traditional rubber/cotton solution may be regarded as an alternative material.

Trends and perspectives

Water beds

According to Fachverband Wasserbett (2010), there may currently be a shift towards a higher sale of the low-end water beds with possible higher prevalence of phthalate plasticiser due to the general financial crisis. One producer estimates that some 40-50% of the world market for water beds is produced with Mesamoll II, and expects that this share will increase.

Air mattresses

One producer informs that "non-6P" PVC film is now also available for air mattress production. This PVC film is without DEHP, DBP, BBP, DiNP, DiDP, DNOP. However it is not possible to say something about the trend.

B.2.2.8 Wallpaper/tapestry made of or coated with PVC

Definition of the product group

The product group covers wall papers or wall coverings made of or coated with plasticised PVC. PVC wallpaper is also called vinyl wall covering, but should not be confused with the thicker wall covering products used for bathroom walls etc. (vinyl flooring style coverings), see section 2.2.1.

Import to and production of articles in EU
Import of wallpaper made of or coated with PVC to EU27 is estimated to be around 4,800 tonnes pr. year, while production in EU27 estimated to be around 294,000 tonnes pr. year

Major producers have confirmed that most wallpaper marketed in the EU is PVC wallpaper produced in the EU. Import from the USA may occur, but is not widespread due to other design preferences.

Plasticisers in use
According to major European producers DINP is today the main plasticiser used in wallpaper/wall covering manufacturing. The use of DOTP has however also been reported, as well as DOA and TXIB for special purposes (secondary plasticisers). All producers contacted reported that they do not use DEHP, BBP, DBP or DIBP in wallpaper.

According to major producers of PVC wallpaper, typical plasticiser concentrations are 25-30%. Based on the statistics presented above the amount of plasticisers used for production in EU may thus be roughly estimated to approx. 60,000 - 90,000 tonnes, which should be regarded as mainly DINP with lesser quantities of DOTH, DOA, TXIB and probably also DEHP and BBP as stated below.

A total DEHP consumption of 10,100 tonnes per year in the EU was estimated for "wall covering" for 2007, based on updated DEHP production statistics in combination with partly older consumption distribution estimates, and import/export statistics. Most of this was used in European production. The term "wall covering" may be used for both PVC wall paper and flooring style "bathroom" vinyl, and thus these product types may both contribute to this consumption estimate (ECHA, 2009a). Based on similar considerations, the total consumption of EU-produced BBP for "packaging films, calendared flooring, wall covering" was estimated at 560 tonnes per year for 2007. Here, import/export were not included in the estimation process, but it is known from producers interviewed in the present study, that import of wall paper/wall covering is minor compared to EU production. The similar ECHA report on DBP (ECHA, 2009c) does not mention wall paper/wall covering as one of the product types, where DBP may be used.

In 2001 four PVC wall papers were analysed for the concentration of phthalates. Two wall papers had a content of DINP and DIDP between 23 and 26 % and the other two had a content of DEHP between 6.9 and 9 % (Danish EPA, 2001).

In the survey from 2010 15 wall papers were analysed for the content of the four phthalates DEHP, DBP, DIBP and BBP. The analyses showed all wall papers had a content of the three of the phthalates (DEHP, DBP and DIBP) in individual concentrations below 0.1 %, but 10 of the wall papers contained DINP. The content of DINP was not quantified. BBP was not detected in any of the analysed wallpapers (Danish EPA, 2010a).

Alternatives available
Producers report that DINP, DINCH, DOA and DOTP are all used by the wall coverings industry, and that there are no wall paper qualities which cannot be produced without DEHP, BBP, DBP and DIBP.

Trends and perspectives
Substitution away from DEHP, BBP, DBP and DIBP seems to have started several years ago. No other information was given by producers as regards trends and perspectives. The case for imports is unknown.

B.2.2.9 Footwear

Definition of the product group
The product group covers sandals and slippers/flip flops made partly or completely of PVC. The group also covers thermo boots for children.

Import to and production of products in EU
Import of the relevant footwear to EU27 is estimated to be around 115,000 tonnes pr. year, while production in EU27 is estimated to be around 92,000 tonnes pr. year

It may be noted that both import and EU27 manufacturing of sandals and slippers are significant. However, as the quantity manufactured is estimated based on the manufacturing value combined with the value/weight relationship for import data, the estimated quantity manufactured could be biased.

Shoes in general
No exact knowledge of the share of shoes containing PVC and the precise average content of PVC in shoes are available. An indication of the share of shoes containing PVC may be obtained from a Swedish study, in which tests of 27 different plastic sandals and slippers purchased in 7 countries (Sweden, Philippines, South Africa, Indonesia, India, Uganda and Tanzania) world wide, was made (Naturskyddsföreningen, 2009). The testing revealed that 18 of the 27 tested shoes corresponding to approx. 67% contained PVC. Of the 18 pair of shoes containing PVC, 17 pair contained classified phthalates (DEHP and/or DBP).

Considering only shoes purchased in the EU 4 pairs of sandals etc. were sold in Sweden. 2 pairs contained PVC and both of these contained DEHP and DBP (Naturskyddsföreningen, 2009). The Swedish investigation revealed no data on the share of PVC compared to other materials and could thus be biased.

Considering an import of 115000 tonnes of shoes of which 50-67% contains PVC, and that half of the weight of each such shoe is PVC, leads to a estimate of a yearly import of PVC to EU with sandals, slippers etc. of approx. 29.-38.000 tonnes.

Thermo boots
There is no known production of thermo boots in Denmark or the EU.

Based on information from Danish importers of thermo boots, it is estimated that the import to Denmark is approx. 330,000-470,000 pair of boots per year. Thermo boots weigh between 600 g (size 24) and 1,000 g (size 32) including lining. Based on this information it is estimated, that the average weight of thermo boots is 800 g excluding lining (and thus the weight of the PVC including phthalates). This results in an import to Denmark of PVC with thermo boots at approx. 260 - 380 tonnes per year. It is difficult to upscale this amount to the European scale because of the different climate conditions.

Plasticisers in use

Sandals, flip-flops etc.

A Swedish investigation by "Naturskyddsföreningen i Sverige" revealed that the PVC in the tested footwear contained up to 23.2% DEHP, up to 9.6% DBP, no BBP, up to 19.4% DNOP, up to 3.2% DINP and up to 4.7% DIDP (Naturskyddsföreningen, 2009).

The investigation furthermore showed that regarding the phthalate content of PVC in shoes there seems to be no difference on in which country the shoes were manufactured (Naturskyddsföreningen, 2009).

In an investigation from the Danish EPA (2010c) 60 plastic sandals were analysed for the content of DEHP, DBP, DIBP and BBP. The sandals were distributed on 20 sandals for adults, 20 sandals for 2-year-olds and 20 sandals for 6/7-year-olds.

10 of the sandals for adults contained one or more of the four phthalates in concentrations above 1 %, the highest concentration found was 46 % of DEHP. Six of the sandals for 2-year-olds contained one or more of the four phthalates in concentrations above 1 % and the highest concentration found was 34.5 % of DEHP. 5 of the sandals for the 6/7-years-old contained one or more of the four phthalates in concentrations above 1 % and the highest concentration found was 46.2 % of DEHP. Other phthalates were found in some of the sandals, but the phthalates were not quantified. Common for all the analysed sandals are that only low concentration of BBP were detected (below 0.1%) (Danish EPA, 2010c).

Thermo boots

According to information from producers, the choice of phthalate varies for thermo boots; one producer uses DINP and DIDP. Another producer uses mainly DEHP together with small amounts of DBP and DIDP.

According to an interview with the laboratory manager from Intertek (world wide group of testing facilities) for toys and hardlines, thermo boots sold in Europe are made of PVC. The most used plasticiser is DEHP and the concentration is in the range of 20-35%. Several other "replacers" are also used together with DEHP (Intertek, 2010) (see below).

Alternatives available

Alternatives available include other materials as well as other plasticisers.

The general experience among purchasing agents is that alternatives to phthalates and PVC are available for all types of products. The main alternative material utilised is polyurethane (PU). Considering alternative plasticisers apart from those listed above, tests of thermo boots indicate that a small fraction of the tested thermo boots contains DINCH, "Citroflex" (ATBC) or "Eastman 168 – 1-4 - disubstituted" (DOTP) (Intertek, 2010).

Trends and perspectives

The market is very diverse and no general trend seems to dominate the market.

B.2.2.10 Bathing equipment (swim-coats/wings/belts and pools)

Definition of the product group

The product group covers all kinds of bathing equipment made of plasticised PVC film or coated fabrics inclusive of pools (inflatable and non-inflatable), swim-coats/wings/belts except items classified as toys, as these are already covered by existing legislation in REACH Annex XVII.

Import to and production of articles in EU
Import of bathing equipment etc. to EU27 is estimated to be around 203,000 tonnes pr. year, while production in EU27 estimated to be around 423,000 tonnes pr. year

It is clear that both import and EU27 manufacturing are significant. Import can be calculated to count for approx. 50% of the quantity manufactured. However, the figures quoted will include several other items than just bathing equipment. Focusing on bathing equipment, other information available - see below - seems to indicate that import is very important and the EU market is dominated by products manufactured in Asia.

In the report (Danish EPA, 2003), it is roughly estimated by the Association of Danish Plastic Manufacturers that about 30% of the products in this category are made of PVC only. Assuming this estimate still to be reliable at least for the import of bathing equipment etc. to EU, it can be assessed that the import will include approx. 60,000 tonnes of PVC.

The knowledge available does not allow for any estimate of the content of PVC in articles manufactured within the EU. The considerations presented in the following are therefore focused on the import only.

It is known that a dominant manufacturer of bathing equipment is the American company Intex, who are producing outside EU (at least regarding the Danish market), but it is estimated that this is also the situation in the EU as a whole perhaps with small local deviations.

Information from dominant suppliers to the Danish market states that only the large inflatable swimming pools and pool covers contain phthalates.

Plasticisers in use
Intertek who performs tests on bathing equipment informs that DEHP is the preferred plasticiser with concentrations in the interval 20-40%. Alternatively 20-30% DINP is used (Intertek, 2010).

For inflatable swimming pools etc. sold in Denmark and the other Nordic countries, a dominant supplier also assumes DEHP to be widely used.

Based on information from Intex and the quantitative estimates presented above it can be roughly calculated that the import of PVC to EU with these products results in an import of approx. 1,800-2,700 tonnes of DINP and 1,500-1,800 tonnes of DEHP (order of magnitude).

The Danish EPA has analysed 12 pieces of swimming equipment and 8 swimming pools in 2010. All analysed products are products that are not considered as toys, and they are analysed for the content of DEHP, DBP, DIBP and BBP. Only one of the 12 analysed swimming equipments had a content of DEHP above 1 %, and contained 33 %. DBP and BBP were not detected in any of the analysed samples. 26 % DEHP was detected in one of the analysed swimming pools. Other phthalates than DEHP, DBP, DIBP and BBP were detected in 7 other pools, but the amount was not quantified. BBP was not detected in any of the analysed pools (Danish EPA, 2010b).

Alternatives available

As mentioned above DINP is alreay used as plasticiser in bathing equipment. According to Intex, the non-orthophthalate alternatives could be DINCH and DOTP among others. No supplementary information to this statement is available.

Trends and perspectives
Intex informs that they will "*continue using the phthalates that we use currently in our products if no restrictions*". Considering Intex to be a dominant manufacturer of bathing equipment supplied to the EU, this statement is of significant importance regarding the trend and perspectives in the EU.

However, while the statement from Intex probably is representative for the general trend on the market, other positions may exist as well.

A dominant supplier to the Danish market thus informs that they will keep on working to remove phthalates from the products.

B.2.2.11 Balls for playing and physical exercises

Definition of the product group
The product group covers balls made entirely of PVC, PVC- film and coated fabrics for playing and physical exercises except items that are toys, as these are already covered by existing legislation in REACH Annex XVII. The articles covered by this proposal are thus different kinds of balls used for training and/or matches and physical exercises for health and fitness related purposes.

Import to and production of articles in EU
Import of balls to EU27 is estimated to be around 47,000 tonnes pr. year, while production in EU27 is estimated to be around 13,000 tonnes pr. year

From these figures it may be noted that the import is significant compared to the quantity manufactured in EU27, as the import counts for almost 4 times the quantity manufactured. The difference may actually be higher, as the definition of balls used in the PRODCOM-statistics for manufacturing in EU is broader and thereby covers more items than the definitions used in the import statistics.

It should also be noted that most balls for soccer today is manufactured in polyurethane, and thus is included in the figures for inflatable balls imported to EU stated in the table.

<u>Balls for fitness</u>
Based on information from suppliers to the Danish market, it is roughly estimated that 25,000 - 35,000 fitness balls are sold in Denmark per year via retail shops. No information is available on the sale via fitness centers and the Internet. As no knowledge is available regarding the use of such balls in other EU countries it is not possible to estimate the total consumption in EU.

<u>Soccer balls</u>
Most soccer balls today are manufactured of polyurethane. Cheap balls as the so called "street soccer balls", however, are often manufactured of PVC. Soccer balls are, however, generally build of several layers of materials. Thus is may be so that even seemingly PU balls contains a layer of PVC.

Soccer balls seem typically to be manufactured in Asia (e.g. Pakistan).

Plasticisers in use
Based on information from the manufacturers European production of large plastic balls seems to be made of PVC without phthalates. However, information on the used plasticisers is confidential.

Regarding balls for fitness several manufacturers confirms that the balls are made of or contain PVC. The plasticisers used are DINP or acetyl-tri-n-butylcitrat (ATBC). DIDP and DIOP are used together with DINP. One manufacturer informs that DEHP may be observed in small concentrations (< 0.1 %). No other data on concentration of plasticisers used has been available.

The Danish EPA has analysed 10 balls for fitness in 2010 for the content of DEHP, DBP, DIBP and BBP. The analyses showed that two of the analysed balls contained DEHP in concentrations above 1 % and that two of the analysed balls contained DIBP in concentrations above 1 %. DINP was detected in five balls, but the amount of the phthalates were not quantified (Danish EPA, 2010b).

For soccer balls made of PVC, one manufacturer informs that the balls do not contain plasticisers as DINP, DNOP, DIDP, BBP, DBP and DIHP, but traces of DEHP (concentrations negligible) may be registered. Another large producer informs that DEHP and DBP are used in very low concentrations (<1%). In both cases no information on the main plasticisers used has been available.

Alternatives available
The alternatives available cover other materials as well as other plasticisers.

Regarding materials for soccer balls, dominant international manufacturers inform that PVC has been permanently replaced by PU as a matter of company policy.

For fitness balls - as stated above - the material being used is PVC, and DINP and acetyl-tri-n-butylcitrat (ATBC) are used as plasticisers. DIDP and DIOP may be used together with DINP.

Regarding large plastic balls - as stated above - the material used is PVC, while alternative plasticisers (no phthalates) are employed.

The conclusion is that alternatives to phthalates or PVC are available for all types of balls.

Trends and perspectives
The general trend seems to be a movement away from PVC and phthalates and in particular classified phthalates. Although it is believed that contact has been made to most of the dominant players on the EU market, it cannot be ruled out that the assessment presented above may not be valid for manufacturers not supplying the Danish market or not known to importers of balls to the Danish market.

B.2.2.12 Other articles

Among other articles partly investigated for this dossier was erasing rubbers and nitrocellulose lacquers for coating of wooden furniture and flooring and erasing rubber.

Erasing rubber

A Danish investigation from 2006 (Glensvig & Pors, 2006) has shown the use of phthalates in erasing rubber made of PVC. The investigation has revealed the content of 32 % DINP/DIDP in one sample of erasing rubber. The sample, furthermore, revealed traces (<1%) of DEHP and DBP.

Another Danish investigation analysed four erasers and found DEHP in concentrations between 17 and 44 % (Danish EPA, 2007).

By the EU's "Rapid Alert System for Non-Food Products" (RAPEX) examples on erasing rubbers with concentrations of DEHP has also been reported. The following examples may be noted (EU 2006):

- Erasing rubber shaped as flowers with strong colours, produced i China and sold in Greece in 2005 contained 43,1 % DEHP.
- Pencils with erasing rubber shaped as animals attached, produced i China and sold in Greece in 2006 contained 25,1 % DEHP.
- Round erasing rubbers produced in China and sold in Greece in 2006 contained 31,5 % DEHP.

Nitrocellulose lacquers, paints and inks

Even though the use in paints and laquers is not included in the proposal for restriction it should be mentioned that the ECHA report (ECHA, 2009c) on DBP mentions nitrocellulose lacquers as one of the product types where DBP is used, and estimates an annual consumption of 160 tonnes for this application. The estimate is based on updated (2007) DBP production statistics in combination with partly older consumption distribution estimates and import/export statistics. The same report mentions that DIBP has application properties very similar to the properties of DBP and may therefore be used to substitute for DBP in most, if not all, of its applications.

The total consumption of DEHP for paints and lacquers has been estimated at 900 tonnes per year in 2007, based on similar considerations (ECHA, 2009a).

In the same manner, the total consumption of EU-produced BBP for paints and inks was estimated at 160 tonnes per year for 2007. The main function of BBP in paints and inks is to give flexibility to prevent the paints/inks from chipping and flaking from the surfaces they are applied to (BBP RAR, 2007), (ECHA, 2009b).

For this study, one producer of nitrocellulose lacquers contacted has replied that they have not used phthalate plasticisers for this purpose since 2003. Today they use DOA instead, at a concentration of 2% in wood finishes.

It has to be underlined, that marketing of the classified phthalates in products intended for private use is not allowed.

As regards a related product group, printing inks, ECHA (2009a) states that according to CEPE, (European Council of producers and importers of paints, printing inks and artists' colours) DEHP, DBP and BBP are no longer used in printing inks by CEPE/EuPIA (European Printing Ink Association) members following their classification as reprotoxic category 2. CEPE covers approximately 85% of this industry in the EU. EuPIA represents close to 90% of the printing ink manufacturers selling in Europe. The substances may, however, be used by some manufactures e.g. in new EU Member States, and one manufacturer of DEHP reported that 2% of the tonnage from the manufacture is used for inks (ECHA, 2009a).

B.2.2.13 Summary

Import to and production of products in EU/plasticisers in use.

The share of the amount of the four phthalates in articles for indoor use and articles to come into contact with human skin or muscous membranes including shoe soles is estimated to constitute 85 % of the total amount of the four phthalates in all articles (ECHA, 2009a-c).

The information presented in the previous sections on plasticisers in use and significance of import to EU for the different fields of application is summarised in table 10 below. The data presented clearly illustrates that the situation differs between the fields of application covered.

Table 10 Plasticisers in use/significance of import to products supply on the EU market; summary

Fields of application	**Plasticisers in use – production**	**Plasticisers in use – import**	**Significance of import to supply**
Flooring of vinyl	DEHP, DBP, BBP, DINP, DIDP, DIHP, DIBP, COMGHA, dibenzoates, DIOP*1	No data	Low
Wires and cables	DEHP, DINP, DIDP, DPHP, TOTM, adipates	No data	Low
Bags, brief-/suitcases and similar	DEHP, DIBP, DBP, DINP, DIDP	no examples of phthalates usage identified *2	Equals EU production
Tablecloth, curtains, shower curtains and similar	DEHP, DBP, BBP, DINP, ATBC, DINCH, DOA, ESBO, DIDP	No data	Low
Carpet tiles/squares	DINP	No data	Low
Water beds- and air mattresses	DINP, ASE, unspecified phthalates	May not differ from EU production	High
Wallpaper/tapestry	DINP, DOTP, DOA, TXIB and maybe DEHP and BBP	No data	Low
Footwear	DEHP, DBP, DNOP, DINP, DIDP	May not differ from EU production	Equals EU production
Bathing equipment	No data	DEHP, DINP	? *3
Balls for playing and physical exercises	DINP, DIDP, DIOP, ATBC, other non-phthalates? DEHP, DBP, DIBP	May not differ from EU production	High
Others - erasing rubber	DEHP, DINP, DIDP*1	May not differ from EU production	No data
Others - lacquers for wooden furniture and flooring	DEHP, DBP, BBP, DOA*1	No data	No data

*1 Information on use of DEHP and other phthalates stated dates back to 2007 and before. The picture may have changed since then.

* 2 no examples of phthalates usage identified; data may only be representative for Danish companies and suppliers to Danish companies.

*3 Statistical data are not consistent with information from market actors. Information from market actors indicates import from Asia to be very significant for bathing equipment.

Looking at the information received on the investigated applications, the 4 classified phthalates are still in use in flooring of vinyl, wires and cables, tablecloth etc., footwear, pool and pool covers, balls for fitness, erasing rubber and lacquers for furniture and flooring.

Some manufacturers, furthermore report presence of classified phthalates in balls in concentrations below 1%. These observations, however, should probably be regarded as contamination rather than intended use.

For other areas no evidence has been found that classified phthalates are still in use, and for these applications the classified phthalates seem to have been substituted by other phthalates such as DINP and by non-phthalate plasticisers.

In this context attention must be paid to that the information collected and presented here generally represents either Danish companies or international companies operating in many countries. The information is not necessarily representative for all markets within EU and for all companies operating in EU.

The information that phthalates are not used anymore within the field of EU production of bags, brief-/suitcase and similar is e.g. probably highly influenced by the fact, that data collection for these products was undertaken in Denmark and that most information was available on school bags, a product group for which significant concern is invested by manufacturers and suppliers to the Danish market in order to eliminate potential concerns to consumers. Therefore, it may well be so that phthalates and even the four phthalates are still used in similar products marketed in other countries in EU or could be imported also to Denmark by minor importers.

Data on plasticisers used in imports are scarce or lacking for products where manufacturing in EU dominates (e.g. flooring of vinyl), as it has not been possible to identify the companies responsible for the import. For the products where import by EU based brands dominate, however, it must be assumed that the material composition of the imported product is determined by the importing company and thus most likely will not differ significantly from products manufactured in EU.

It is important to underline, that the data available has not enabled a full quantification of the four phthalates present in imports and EU manufacture of the products covered.

A major constraint in this context has been that statistical data for several product groups are not sufficiently detailed to provide reliable knowledge on volume produced or imported (particular the case for the PRODCOM database on EU-production). Also, many European trade organisations seem to have relatively little knowledge about the substances used in products within their market segment, and are therefore not able to provide significant information on the use of plasticisers in products produced in, or imported to, EU.

Alternatives available

For all fields of application considered, alternatives to classified phthalates are available on the market. The alternatives available may include other plasticisers as well as other types of materials. Reference is made to table 11, in which the information presented in the previous sections on alternatives available for the different fields of application is summarised.

The dominant alternatives to classified phthalates reported used for the covered product groups are DINP and DIDP, but also other ortho-phthalates as DIHP, DPHP and DIOP. Sometimes several of these are used in combination. It is, however, relevant to note that for most fields of application non-orthophthalate plasticisers such as the terephthalate DOTP, and other plasticisers types such as

DINCH, ASE, ATBC, COMGHA and dibenzoates are actually in use or being considered by manufactures as realistic alternatives to orthophthalates.

Table 11. Available alternatives to the four classified phthalates, summary

Fields of application	**Alternatives available**
Flooring of vinyl	DINP, DIDP, DIHP, DIOP, COMGHA, dibenzoates
Wires and cables	DINP, DIDP, DPHP, TOTM, adiapates Other materials: PE, silicon rubber
Bags, brief-/suitcases and similar	Alternatives are available for materials as well as plasticisers - little information on alternatives actually employed
Tablecloth, curtains, shower curtains and similar	DINP, ATBC, DINCH, DOA, ESBO. Other materials: Polyester, EVA, PEVA
Carpet tiles/squares	DINP, DOTP, DIHP, DINCH, non-PVC materials (like bitumen and textiles)
Water beds- and air mattresses	DINP, ASE, "non-classified phthalates"[1]
Wallpaper/tapestry	DINP, DOTP, DOA, DINCH
Footwear	DINP, DINCH, ATBC etc.
Bathing equipment	DINP, DINCH, DOTP
Balls for playing and physical exercises	DINP, DIDP, DIOP, ATBC, other non-phthalates?
Others - erasing rubber	DINP, DIDP
Others - lacquers for wooden furniture and flooring	DOA

1. "non-classified phthalates" means that DEHP, DBB and DBP are not used

B.2.3 Uses advised against by the registrants

As far as the dossier submitter knows there is in the registration dossiers no advice against any specific uses in articles except for those restrictions already in place.

B.2.4 Description of targeting

The proposed ban is targeted at articles that the consumers and workers can either come in direct skin or mucous contact with or articles that contribute to the exposure to phthalates from the indoor environment. The ban is directed at all relevant articles; most of the articles are made of PVC as the dominat uses of the phthalates are as softeners in PVC.

Some groups of articles are not covered by this proposal as they are already covered by other legislation; especially toys are relevant, but there is already a ban for this use for three of the four phthalates.

The argumentation for covering the above mentioned articles is based on the exposure of both children and adults to the combined exposure from the four phthalates coming from all sources including the exposure from indoor environment and food.

B.3 Classification and labelling

B.3.1 Classification and labelling in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) and Directive 67/548/EEC

Table 12 . Classification and labelling of the four phthalates.

Substance	CAS no.	Classification and labelling according to Regulation 1272/2008		Classification and labelling according to Dir. 67/548/EEC	
		Hazard class and category codes	Hazard statement codes	Classification	Labelling
DEHP	117-81-7	Repr. 1B	H360-FD	Repr. Cat. 2; R60-61	T R: 60-61 S: 53-45
BBP	85-68-7	Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H360-Df H400 H410	Repr. Cat. 2; R61 Repr. Cat. 3; R62 N; R50-53	T; N R: 61-62-50/53 S: 53-45-60-61
DBP	84-74-2	Repr. 1B Aquatic Acute 1	H360-Df H400	Repr. Cat. 2; R61 Repr. Cat. 3; R62 N; R50	T; N R: 61-50-62 S: 53-45-61
DIBP	84-69-5	Repr. 1B	H360-Df	Repr. Cat. 2; R61 Repr. Cat. 3; R62	T R: 61-62 S: 53-45

B.4 Environmental fate properties

B. 4.1 Hydrolysis DEHP

The following is from a registration dossier on DEHP:
The studies on hydrolysis are summarised in the following table:

Table 13 Overview of studies on hydrolysis.

Method	***Results***	***Remarks***	***Reference***
no data: Value from Wolfe et al.(1980), who measured alkaline hydrolysis rate constants to monoester.	*Half-life (DT50):* *t1/2 (pH 7): 2000 yr at 25 °C*	*4 (not assignable) weight of evidence experimental result* ***Test material (EC name): bis(2-ethylhexyl)***	*Staples C.A., Peterson D.R., Parkerton T.F. and Adams W.J. (1997)*

		phthalate	
no data, data reported from Callahan et al. (1980) (US EPA report)	*Half-life (DT50): t1/2 (pH 7): 2000 yr at 25 °C*	*4 (not assignable) weight of evidence experimental result* ***Test material (EC name): bis(2-ethylhexyl) phthalate***	*Giam CS, Atlas E, Powers MA, Leonard JE (1984)*

Discussion

The following information is taken into account for any hazard / risk / persistency assessment:

On basis of two experimental measures from literature a very slow hydrolysis of DEHP to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol is expected.

An estimated half-life of approximately 2,000 years has been reported

B. 4.2 Hydrolysis DBP

The following is from the EU RAR on DBP:

A test on the hydrolysis potential of DBP indicated that at pH 4.0 and 7.0 DBP was found to be stable, i.e. less than 10% hydrolysis after 5 days. At pH 9.0 and a temperature of 50 ℃ a half-life time of 65.8 hours was reported. These results are in line with the RIVM-conclusion (RIVM, 1991) that the contribution of hydrolysis to the overall environmental degradation of phthalate esters, including DBP, is expected to be low.

B. 4.3 Hydrolysis DIBP

The following is from a registration dossier on DIBP:

The studies on hydrolysis are summarised in the following table:

Table 14 Overview of studies on hydrolysis

Method	***Results***	***Remarks***	***Reference***
Procedure as described in Wolfe et al. (1976) Agric. Food Chem., 24, 1041 and Wolfe et al (1977) Environ. Sci & Tech., 11, 88 Sodium hydroxide induced hydrolysis is monitored by GLC on samples.	*Half-life (DT50): t1/2 : at 30 °C; Rate constant: 0.0014 ; Type: second order (Units: M-1.s-1)*	*2 (reliable with restrictions) weight of evidence experimental result* ***Test material (EC name): diisobutyl phthalate***	*Wolfe, N., Steen, W. and Burns, L. (1980)*

DIBP is not expected to undergo hydrolysis in the environment due to a lack of hydrolysable functional groups.

B. 4.3 Hydrolysis BBP

The following is from a registration dossier on BBP:

Hydrolysis and phototransformation in water are expected to be insignificant.

B.5 Human health hazard assessment

The four phthalates DEHP, DBP, DIBP and BBP are all classified as toxic to reproduction in category 1B. These four phthalates are considered to have endocrine disrupting effects with similar mode of action as described in section B.1.4 Justification for grouping.

B.5.1 Toxicokinetics

To be able to estimate the risk from exposure data by different routes, internal dose estimates need to be established for exposure estimates as well as for no-effect level estimates. Table 15 lists the applied absorption fractions used in calculation of internal doses in human exposure estimates as well as in the determination of no-effect levels in animal studies. The absorption fractions for humans are all assumptions of the absorption.

Table 15 Absorption fractions for calculation of internal doses as indicated in EU risk assessment reports.

	Absorption fraction, oral	**Absorption fraction, dermal**	**Absorption fraction, respiratory**
DEHP	50% rats, all ages 50% adult humans 100% infants/children	5% human, all ages	75% adults 100% infants/children
DBP and DiBP	100% (exp animals and humans)	10% human, all ages	100% human, all ages
BBP	100% human, all ages	5% human, all ages	100% human, all ages

For DEHP, an oral absorption fraction of 50% has been determined in rat and monkey studies, whereas human data are not available. The EU RAR for DEHP uses a 100% oral absorption fraction for children as these are considered susceptible and an oral absorption fraction of 50% for adults. The absorption fraction of 100% in infants/children thus includes a protective factor that is not used for animal studies on developing animals. The current report uses similar absorption fractions as the EU RAR for DEHP, DBP and BBP, although data on recently detected metabolites of DEHP show an oral absorption around 70% in adult humans (EU RAR, 2008a). Due to similarities between DBP and DIBP it is assumed that DIBP has the same absorption fraction as DBP.

B 5.2 Acute toxicity

Not relevant

B 5.3 Irritation

Not relevant

B 5.4 Corrosivity

Not relevant

B 5.5 Sensitisation
Not relevant

B 5.6 Repeated dosed toxicity
Not relevant

B 5.7 Mutagenicity
Not relevant

B 5.8 Carcinogenicity
Not relevant

B 5.9 Toxicity for reproduction

Table 16 lists studies on anti-androgenic or reproductive effects of the four phthalates. A derived no effect level (DNEL) for consumers and the general public is listed based on the available literature data. DNEL calculation uses an uncertainty factor of 2.5 for interspecies differences; an allometric scaling factor of 4 for rats and 7 for mice; a factor of 10 for intraspecies differences; and a factor of 3 as extrapolation from LOAEL to NOAEL, if no NOAEL is set in the relevant study (ECHA, 2008). From the studies described below and given in Table 16, one NOAEL is selected for each phthalate and used for the cumulative risk assessment. When selecting NOAELs for cumulative risk assessment it is considered important that the studied effect is relevant to an anti-androgenic mode of action and that the study is considered valid and generally "accepted" by either the EU risk assessment report (EU RAR) or by EFSA opinions. It should be noted that NOAELs are based on different types of specific effects assumed to have the same mode of action.

The selected NOAELs for DEHP, BBP and DIBP are supported by several studies leading to NOAELs within the same range. For DBP, the studies listed in Table 16 show a large variation in NOAELs/LOAELs. For the four phthalates, the following studies have been considered critical:

- DEHP: Studies by Wolfe & Layton (2003), Christiansen et al. (2010) and Andrade et al. (2006) are critical for the NOAEL selection. Andrade et al., 2006, described increased incidence of cryptorchidism from 5 mg/kg bw/day in male rats exposed to DEHP from GD 6 to PND 21, with a NOAEL of 1.215 mg/kg bw/day. Christiansen et al. (2010), found reduced anogenital distance and increased nipple retention in male rats perinatally (GD7 to PND 16) exposed by gavage to 10 mg DEHP/kg bw/day and above, with a NOAEL of 3 mg/kg bw/day (Christiansen et al., 2010). A NOAEL of 4.8 mg/kg bw/day was found in the study by Wolfe and Layton, 2003, who observed testicular toxicity in offspring exposed to 14 mg DEHP/kg bw/day and above in a multigeneration study with dietary exposure (Wolfe & Layton, 2003). When combining NOAELs and LOAELs from these three studies, the highest NOAEL is 4.8 mg/kg bw/day based on the study by Wolfe & Layton and the lowest LOAEL is 5 mg/kg bw/day based on the findings in the study by Andrade et al. (2006). If the study by Andrade et al. (2006) is not taken into consideration (due to the observation of cryptorchidism in only few animals), the lowest LOAEL would be 10 mg/kg bw/day based on the study by Christiansen et al., 2010, and that would not change the NOAEL determination from the EU RAR. The **NOAEL of 4.8 mg/kg bw/day** is selected for cumulative risk assessment and was also used by the EU RAR and EFSA. This is based on testicular effects that can be attributed to an antiandrogenic mode of action.

- Details of the key studies:

- The study by Wolfe & Layton (2003), is described in detail in EU RAR for DEHP and considered acceptable and used as a key study for selection of overall NOAEL by the EU RAR. The study by Wolfe & Layton (2003) is also used as the critical study in the registration dossier for DEHP. Details on the study by Wolfe & Layton (2003) are presented in the EU RAR (page 413 to 424 of the EU RAR) presented in annex 2. In brief, this study is a multigeneration study in Sprague-Dawley rats exposed to dietary concentrations of DEHP of 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm of DEHP (n=17 males and females), corresponding to 0.1, 0.47, 1.4, 4.8, 14, 46 and 359 mg/kg bw/day in F2 animals. It should be noted that the control group received 1.5 ppm of DEHP, as this was the amount of DEHP found in control feed. Testicular effects were most prominent in F1 and F2 animals, and a NOAEL of 100 ppm corresponding to 48 mg/kg bw/day in F2 animals was determined by the EU RAR as the critical NOAEL for testicular toxicity and developmental (testicular) toxicity (EU RAR page 424). For a comprehensive discussion of this NOAEL selection is referred to the relevant pages of the EU RAR, annex 2.

- The study by Christiansen et al. (2010), is not described in the EU RAR or in the registration dossier for DEHP. Christiansen et al. (2010), describes two non-guideline, non-GLP studies with exposure of time-mated Wistar rats from GD 7 to PND 16 by gavage with DEHP in corn oil. Study 1 included 16 mated dams in the control group and 8 mated dams per group in six exposure groups receiveing either 10, 30, 100, 300, 600 or 900 mg/kg bw/day of DEHP. Study 2 included 16 mated dams in the control group, 16 mated dams receiving 3 mg/kg bw/day of DEHP, and 8 mated dams per group receiveing either 10, 30, or 100 mg/kg bw/day of DEHP. A number of reproductive endpoints were investigated postnatally and at PND 16. The critical effect of the combined evaluation of the two studies were effects on anogenital distance and nipple retention in males, as the anogenital distance was significantly decreased and the number of nipples significantly increased at 10 mg/kg bw/day of DEHP with a NOAEL of 3 mg/kg bw/day. At the same dose (10 mg/kg) and above, decreased weights of ventral prostate and levator ani/bulbocavernosus muscle were observed, though these effects did not show a clear dose-response relationships. The findings in the study by Christiansen et al. (2010), is considered acceptable and supports the findings by Wolfe & Layton (2003).

- Andrade et al. (2006), describes a study on in utero and lactational exposure of Wistar rats to DEHP at low and high doses by gavage, and showed effects on daily sperm production from 15 mg/kg bw/day and a low, but increased incidence of cryptorchidism at 5 mg/kg bw/day. Pregnant Wistar rats were gavaged from GD 6 to PND 21 with 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135 and 405 mg DEHP/kg bw/day (n=11 to 16 litters per dose). Effects on hormone levels were seen at low doses, but did not exhibit dose-response relationships. In males exposed to 1.215 mg/kg bw/day and at doses from 15 mg/kg bw/day and above (i.e. not at 5 mg/kg bw/day), daily sperm production was reduced compared to controls from the same study and compared to historical controls. The authors concluded a LOAEL of 15 mg/kg bw/day for this effect. Three animals exposed to 5, 135, and 405 mg/kg bw/day of DEHP, respectively, had undescended testes (chryptorchidism). The authors concluded a NOAEL of 1.215 based on cryptorchidism despite the low number of affected animals, as chryptorchidism is less common in Wistar rats

compared to other rat strains. However, as this LOAEL of 5 mg/kg bw/day is above the selected NOAEL of 4.8 mg/kg bw/day in the study by Wolfe and Layton, 2003, including this finding does not affect this overall NOAEL selection.

- Other studies on DEHP were considered for the NOAEL selection but were not considered critical. A large number of reproductive, developmental and mechanistic studies published up to 2003 are described in the EU RAR (Table 4.58 "Important reproductive studies with DEHP in laboratory animals" in EU RAR), but as the EU RAR did not consider these studies critical with repect to reproductive effects of DEHP, these studies were not evaluated further. Additionally, the registration report for DEHP includes the following studies, which were not considered critical: Two studies by Howdeshell et al. (2008), and Hannas et al. (2011), are included in Table 14 and describe effects of DEHP on fetal testosterone production in rats at doses from 300 mg/kg bw/day. These are some of several mechanistic studies describing inhibitory effects of DEHP on fetal testosterone at higher levels than those inducing other male reproductive effects. Hannas et al. (2011) compared the exposure of SD and Wistar rats to 0, 100, 300, 500, 625, 750 or 875 mg DEHP /kg/day from GD 14 to 18. Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Despite differences in testosterone production values in the two strains, the same response was seen, i.e. a decrease in testosterone production at 300 mg/kg bw/day and above with a NOAEL of 100 mg/kg bw/day. Howdeshell et al. (2008), found that DEHP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). In this study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of DEHP from GD 8 to 18 by gavage in corn oil (n=5 to 8 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for DEHP and the other tested phthalates (BBP, DBP, and DIBP) from 300 mg/kg bw/day and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/day.

- The following studies published after 2003 all included doses higher than 10 mg/kg bw/day (=LOAEL) and were therefore not taken into consideration for NOAEL determination: Tanaka et al. (2003), Tanaka et al. (2005), Gray et al. (2009), Wilson et al. (2007), Howdeshell et al. (2007), Shirota et al. (2005), Borch et al. (2005), Tomonari et al. (2006), Cammack et al. (2003), Wilson et al. (2004), Liu et al. (2008).

- DBP: Lee et al., 2004, found reduced spermatocyte development in prepubertal rats and mammary gland changes in adult male rats perinatally (GD 15 to PND 21) exposed to 2 mg DBP/kg bw/day and above via the diet. No NOAEL was determined. In that study, anogenital distance was reduced and nipple retention was increased in males at 1000 mg DBP/kg bw/day with a NOAEL of 200 mg/kg bw/day. The EU RAR on DBP from 2003 uses an overall LOAEL of 52 mg/kg bw/day for embryotoxicity based on a study by Wine et al., 1997. In 2005, EFSA concluded a Tolerable Daily Intake (TDI) of 0.01 mg/kg bw/day for DBP based on delayed

germ cell development and male mammary gland changes in the study by Lee et al., 2004 (see annex 3).

It is reasonable to regard the observed mammary gland effects as anti-androgenic. A 28-day study on the androgen receptor antagonist flutamide showed a dose-related induction of lobular atrophy in male mammary glands (Toyoda et al., 2000). The authors suggested that the observed lobular atrophy of the mammary glands may be due to an anti-androgenic action on acinar cells, as also seen in *in vitro* studies (Toyoda et al., 2000; Boccuzzi et al., 1995; Sourla et al., 1998). The same mechanism of action may apply to the lobular atrophy observed with DBP in the study by Lee et al., 2004.

As the observed effects of DBP on mammary gland and testes are considered anti-androgenic, and as EFSA has chosen to change the TDI in favor of the study by Lee et al., the **LOAEL of 2 mg/kg bw/day** is suggested for use in the current cumulative risk assessment.

Details of the studies by Lee et al. (2004) can be found in the EFSA opinion from 2005 and is cited below (citation in *italics*): *In a recent developmental toxicity study (Lee et al., 2004) with exposure during the period from late gestation (Gestational day 15) to the end of lactation on postnatal day 21 (PND 21), maternal rats were given DBP at dietary concentrations of 0, 20, 200, 2000 and 10000 mg/kg. Major results of this study are summarised below.*

At PND 2, anogenital distance was significantly reduced in 10000 mg/kg male offspring. At PND 14, the incidence of retained nipples/areolae was increased in all treated male offspring compared with controls but the increase was only significant at 10000 mg/kg. At PND 21, in males, reduction of spermatocyte development as manifested by a decreased number of spermatocytes was observed from 20 mg/kg with dose-dependent increased incidence or/and severity. A significant increase in scattered foci of aggregated Leydig cells was observed at 2000 mg/kg and 10000 mg/kg. In the epididymis, significantly decreased ductular cross sections, indicating reduced coiling, were observed at 2000 and 10000 mg/kg. In the mammary glands, dilatation of alveolar buds and/or ducts was seen in male offspring from 20 mg/kg with low incidence but not achieving statistical significance in any group. In female offspring, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 20 mg/kg with a statistically significant increase at 20, 200, 2000 and 10000 mg/kg ($P<0.05$). At postnatal week 11 (PNW 11), in males, loss of germ cell development was significant at 2000 mg/kg and above. This lesion differed markedly in severity between animals. Significant increases in vacuolar degeneration in the mammary glands of males was present from 20 mg/kg but with similar incidence and qualitative gradation of change across the dose groups (End of citation from EFSA opinion 2005).

- Other studies on DBP were included in the NOAEL determination by EFSA but were not considered critical. These studies include developmental and reproductive studies described in detail in the EU RAR (page 86 to 98, see annex 4): Lamb et al. (1987), Morrisey et al. (1989), Gray et al. (1999), Mylchreest et al. (2000), NTP (1995), Wine et al. (1997). The EU RAR also describes the following studies that were not discussed in the EFSA opinion from 2005: Nikoronow et al. (1973), IRDC (1984), Hamano et al. (1997), Shiota et al. (1980), Ema et al. (1993), Mylchreest et al. (1998), Mylchreest et al. (1999) (see EU RAR for precise references). The registration report for DBP quotes strictly the EU RAR regarding reproductive and developmental toxicity and therefore describes the same studies. The EU RAR determines a LOAEL of 52 mg/kg bw/day based embryotoxicity in the study by NTP (1995)/Wine et al. (1997). As embryotoxicity is not considered an anti-androgenic effect, this LOAEL is not considered for the current cumulative risk assessment. The lowest LOAEL reported in the EU RAR and related to anti-androgenicity is 100 mg/kg bw/day in a

study by Mylchreest et al. (1999), in which delayed preputial separation was seen at the lowest dose of 100 mg/kg bw/day. The study describtion from the EU RAR follows below (Citation from EU RAR page 95 is in *italics*):

In a follow-up study of Mylchreest et al. (1999) DBP was shown to disrupt the androgenregulated male sexual differentiation during prenatal exposure, without interacting directly with the androgen receptor, as does flutamide, a known antiandrogen. At the highest dose-level of 500 mg/kg bw (in corn oil), given orally by gavage to pregnant rats during day 12-21 of gestation, one dam showed weight loss after day 18 of pregnancy and delivered dead and moribund fetuses. At all dose levels (100, 250 and 500 mg/kg bw) delayed preputial separation in F_1 males (killed at sexual maturity at the age of 100-105 days) was seen. At the lowest dose level of 100 mg DBP/kg bw this delay (of 2 days) was attributable at least in part, to one markedly affected litter. Furthermore malformations of the (F_1) male reproductive tract were observed at 250 and 500 mg/kg bw, i.e. retained thoracic nipples and decreased anogenital distance. In addition, at 500 mg/kg bw hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of seminiferous epithelium and interstitial cell hyperplasia (5 animals from 2 litters) of the testis were seen. Interstitial cell adenoma occurred at 500 mg/kg bw in 2 males (in one litter). In F_1 females no abnormal uterine or vaginal development or kidney agenesis were seen. In contrast to flutamide, DBP caused a low incidence of prostate agenesis and hypospadias with no vaginal pouch.

- A second follow-up study by Mylchreest et al. (2000), is mentioned by EFSA (2005), but not by the EU RAR from 2003. In this study a NOAEL of 50 and a LOAEL of 100 mg/kg bw/day was determined based on nipple retention in male pups at 100 mg/kg bw/day and above. This study examined exposure of pregnant CD rats to DBP by gavage from GD 12 to 21 at the doses of 0, 0.5, 5, 50, 100 or 500 mg/kg bw/day. Nipple retention was the only effect observed at 100 mg/kg bw/day, and at 500 mg/kg bw/day decreased anogenital distance of males, hypospadias and absence or malformations of epididymis, vas deferens, seminal vesicles and ventral prostate was seen together with decreased withts of male reproductive organs and histological changes in testes.
- A number of reproductive/developmental studies have been published after the EU RAR from 2003. A study by Zhang et al. (2004), detected a NOAEL of 50 mg/kg bw/day based on decreased anogenital distance of males and effects on male reproductive organs and sperm production of rats exposed to 250 or 500 mg/kg bw/day of DBP in utero and during lactation (GD 1 to PND 21).

- A number of reproductive, developmental and/or mechanistic studies applying large doses of DBP are not described here, as these were not considered relevant for NOAEL determination (Ryu et al., 2008, Jiang et al., 2007 and more). Among the mechanistic studies are dose-response studies on the inhibitory effect of DBP on fetal testosterone production:
    - Howdeshell et al. (2008), described that DBP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). In this study, pregnant Sprague-Dawley rats were exposed to 33, 50, 100, 300, or 600 mg/kg bw/day of DBP from GD 8 to 18 by gavage in corn oil (n=3 to 4 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related

decreases in testosterone production was seen for BBP and the other tested phthalates (DIBP, BBP, and DEHP) from 300 mg/kg bw/day and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/day.

- Lehmann et al. (2004) found decreased fetal testosterone concentration in rats exposed to DBP at doses from 50 mg/kg bw/day with a NOAEL of 10 mg/kg bw/day. In that study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of DBP from GD 12 to 19 by gavage in corn oil (n=1 to 4 litters per group, analysis of testosterone in 3-4 males per litter). Testosterone concentration was measured in testes of 19 day old fetuses.

- DIBP: Few reproductive toxicity studies have been published on this compound. Sallenfait et al. (2008), found reduced anogenital distance and increased nipple retention in male rats perinatally (GD 12 to 21) exposed by gavage to 250 mg DIBP/kg bw/day with a **NOAEL of 125 mg/kg bw/day**. A study by Howdeshell et al. (2008), is included in Table 14 and describes effects of DIBP on fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). When combining NOAELs and LOAELs from these studies, the highest NOAEL is 125 mg/kg bw/day and the lowest LOAEL is 250 mg/kg bw/day, both values from the study by Saillenfait et al. (2008). For DIBP, no NOAEL or LOAEL values have been suggested by expert groups, but a LOAEL of 125 mg/kg bw/day for DIBP is used in the registration report for DIBP based on histological changes in testes observed at all doses. The LOAEL of 125 mg/kg bw/day can be considered rather conservative, as this is based on the finding of severe seminiferous tubular atrophy in one animal mainly (see description of study below). The NOAEL of 125 mg/kg bw/day from the study by Saillenfait et al. (2008) is selected for the current risk assessment as it is based on anti-androgenic effects comparable to those seen with the other phthalates. If the 125 mg/kg bw/day is instead used as a LOAEL (as in the registration dossier for DIBP), the NOAEL combining all relevant studies would be 100 mg/kg bw/day based on the study by Howdeshell et al., 2008. The use of this alternative NOAEL would in the end lead to a 20% lower DNEL and 20% lower RCR values.

  - Details of the studies by Saillenfait et al. (2008) and Howdeshell et al. (2008):
    - Saillenfait et al. (2008), describes a study on exposure of pregnant Sprague-Dawley rats from GD 12 to 21 by gavage to 0, 125, 250, 500, or 625 mg/kg bw/day of DIBP. Reduced male neonatal anogenital distance and an increased number of nipples in males were observed from 250 mg/kg bw/day exhibiting clear dose-response relationships. It is noted that non-parametric statistical analyses were applied to the data and did not include body weight in the statistical analysis, but the finding that no significant change in body weight was apparent at 250 mg/kg bw/day supports the conclusion that the change in anogenital distance is real and not a cause of decreased body weight. At higher doses, delays in preputial separation and incidence of malformations (hypospadias, cleft prepuce and undescended testes) were observed in young adulthood and histological changes of testes were observed in adulthood. The observations of histological changes of testes was most marked at 500 and 625 mg/kg bw/day, but mild/infrequent effects were also seen at the two lowest doses. Two of 24 control males had tubular degeneration grade 1 (of 5 grades), whereas 2 of 20 males exposed to 125 mg/kg bw/day of DIBP had tubular degeneration at grade 2 and grade 5, respectively, and 7 of 28 males exposed to 250 mg/kg bw/day of DIBP had tubular degeneration at grade 1 to grade 5. No statistical analysis is presented. The registration dossier for DIBP uses these findings to determine a LOAEL of 125 mg/kg bw/day for DIBP. However, the observation of one animal

with grade 5 tubular degeneration could be a chance finding, whereas the higher number of lesions found in animals exposed to 250 mg/kg bw/day of DIBP is more likely to be an effect of dosing. A LOAEL of 125 mg/kg bw/day would thus be considered conservative, whereas a LOAEL of 250 mg/kg bw/day is less conservative regarding this endpoint.

  - Howdeshell et al. (2008), described that DIBP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). In this study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of DIBP from GD 8 to 18 by gavage in corn oil (n=5 to 8 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for DIBP and the other tested phthalates (BBP, DBP, and DEHP) from 300 mg/kg bw/day and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/day.
  - Hannas et al. (2011), describes a study in which pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of DIBP from GD 14 to 18 by gavage (n=3 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for DIBP and the other tested phthalates (DEHP and DIHP (diisohexyl phthalate)) from 300 mg/kg bw/day and above (NOAEL 100 mg/kg bw/day), and for DINP from 500 mg/kg bw/day.

- Other studies on DIBP were considered for the NOAEL selection but were not considered critical. The described effects on male anogenital distance and fetal testosterone production confirm findings in a study by Borch et al. (2006), showing decreased anogenital distance and decreased testicular testosterone production and –content in fetal male Wistar rats exposed to 600 mg/kg bw/day of DIBP from GD 7 to 21. The registration report for DIBP also includes the following studies, which all applied oral doses at or above the LOAEL of 250 mg/kg bw/day and were therefore not taken into consideration for NOAEL determination: Saillenfait et al. (2006), Boberg et al. (2008), and Zhu et al. (2010). Furthermore, the registration report for DIBP presents a study on DBP (NTP 1995) and justifies use of read-across for effects on fertility; however, this study is not used for NOAEL/LOAEL determination.

- BBP: Tyl et al. (2004) found a NOAEL of 50 mg/kg bw/day due to reduced anogenital distance in male rats exposed to the next dose of 250 mg BBP/kg bw/day. In a study by Nagao et al. (2000), a NOAEL of 100 mg/kg bw/day was determined due to reproductive organ effects and reduction of absolute anogenital distance in males at the next dose of 500 mg/kg bw/day. The EU Risk assessment report from 2007 uses both NOAELs, i.e. a NOAEL of 50 for developmental effects in the study by Tyl et al. and a NOAEL of 100 for effects on fertility and reproductive organs in the study by Nagao et al. A **NOAEL of 50 mg/kg bw/day** is selected here, as this level is used for developmental effects in the EU risk assessment, and this is based on an anti-androgenic endpoint. Both studies were two-generation studies with dietary exposure. The registration dossier includes a two-generation study not reported in the EU RAR and performed by Aso et al. (2005). This study revealed decreasing AGD in male offspring in all doses from 100 mg/kg bw/day of BBP and no NOAEL was determined. Combining this LOAEL of 100 mg/kg with the results of the study by Tyl et al. (2004) and Nagao et al. (2000),

an overall **NOAEL of 50 mg/kg bw/day** can be determined together with a LOAEL of 100 mg/kg bw/day.

- The study by Aso et al. (2005), is a two-generation study with exposure of male and female Crj:CD Sprague Dawley IGS rats to BBP by gavage at doses of 0, 100, 200, or 400 mg/kg bw/day. BBP was administered starting at 5 weeks of age for the F0 parents and 3 weeks of age for the F1 parents for 10 weeks prior to mating, and continued through weaning. Effects in male offspring are summarized here: F1 males had significantly lower body weights from 100 mg/kg bw/day and lowered epididymal weights and increased liver weight from 200 mg/kg bw/day, and reduced seminal vesicle weights and increased thyroid weights at 400 mg/kg bw/day. Aplasia and/or dysplasia and small epididymes and testes were seen at 400 mg/kg, and softening testes were seen at all doses. Histological changes were seen starting at 100 mg/kg bw/day, including atrophy of testicular seminiferous tubules, decreased spermatozoa and residual germ cells in epididymal lumina. Atrophy of seminiferous tubules and hyperplasia of Leydig cells in the testes were significant ($p<0.05$) at 400 mg/kg bw/day. The F2 male offspring had decreased AGD at all doses when corrected by division with the cube root of body weights. The NOAEL was therefore <100 mg/kg bw/day.Details of the studies by Tyl et al. (2004), and Nagao et al. (2000), can be found in the EU RAR together with argumentation for the selection of these two studies as critical for determination of NOAEL for the EU RAR (EU RAR page 167-172, see annex 5).

The following is a summary of these two studies from the EU RAR (citation in italics):

Regarding toxicity to reproduction, fertility as well as developmental studies are available. When taking the available data base into account a NOAEL at 100 mg/kg bw/day for effects on the reproductive organs/fertility from a 2-generation study in rats is used in the risk assessment (Nagao et al., 2000). The NOAEL is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day. In this two-generation study BBP was administered by gavage (0, 20, 100 and 500 mg/kg bw/day) to Sprague-Dawley rats. The results were as following; a significant reduction in fetal body weight was reported at 100 and 500 mg/kg bw/day on pnd 0. Furthermore, in male offspring (preweanling rats) a reduction in AGD (absolute), testis weight, epididymis weight, decreased FSH level and number of spermatogonia and spermatocytes in the seminiferous tubules was reported at 500 mg/kg bw/day. In postweanling rats at 500 mg/kg bw/day a decreased body, testis and epididymis weight was reported. Furhtermore, at 500 mg/kg bw/day, a delay in preputial separation in males, decreased testosterone and LH levels and increased incidence of testicular athrophy with decreased number of germ cells in the seminiferous tubules and decreased number of sperm in the epididymis was reported. In another recent 2-generation study (Tyl et al., 2004) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations was reported (53.33% compared to 0% in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study. For development a NOAEL at 50 mg/kg bw/day for offspring is used in the risk assessment (Tyl et al., 2004). This NOAEL value is based on a dose-related significant

reduction in absolute and adjusted AGD in both F1 and F2 offspring from 250 mg/kg bw/day. At the next higher dose, 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae was reported. At weanling in F1 and F2 offspring a significant reduction in testis weight was reported. At post natal day 21 necropsies the percentage of males with reproductive tract malformations (RTM) were significantly increased in the F1 and F2 offsprings, and at adult necropsies the percentage of males with RTM were significantly increased in the F1 offspring (F2 offspring was not evaluated as adults). In F1 parental male a significant decrease in the testis, epididymis, prostate and seminal vesicle weight was reported (not evaluated in the F2 generation). The NOAEL for maternal toxicity was 750 mg/kg bw/day and was based on organ weight changes (liver and kidney) and histopathological lesions graded as minimal in the liver at 750 mg/kg bw/day. In this 2-generation study BBP was administered in the feed at doses of 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day (end of citation from EU RAR for BBP page 213-214).

- Howdeshell et al. (2008), described that BBP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/day (NOAEL 100 mg/kg bw/day). In this study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/day of BBP from GD 8 to 18 by gavage in corn oil (n=4 to 9 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old fetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for BBP and the other tested phthalates (DIBP, DBP, and DEHP) from 300 mg/kg bw/day and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/day.

- Other studies on BBP were included in the NOAEL selection but were not considered critical. These studies include the following reproductive and developmental studies described in detail in the EU RAR: Agrawal et al. (1985), Piersma et al. (2000), NTP (1997), Piersma et al. (1995), Monsanto (1993), Lake et al. (1978), Hammond et al. (1987), NTP (1990), Parks et al. (1999), Saillenfait et al. (2003), Gray et al. (2000), Ema et al. (1990, 1991, 1992 (a, b, c), 1993, 1994, 1998, 2002), Sharpe et al. (1995), Ashby et al. (1997), TNO (1998 (a, b)), Bayer (1998) (see EU RAR for precise references). Additionally, the registration report for BBP includes a study by Field (1989), which was not considered critical as doses were above the LOAEL of 250 from the study by Tyl et al. (2004): Field (1989). A number of reproductive/developmental studies have been published after the publications listed in the EU RAR (after 2004). The following studies published after 2004 included only doses above the overall LOAEL of 100 mg/kg bw/day and were not considered further for NOAEL determination: Hotchkiss et al. (2004), Liu et al. (2005), Martin et al. (2008), Rider et al. (2009), Kwack et al. (2009), and Moral et al. (2011).

Table 16 NOAEL, LOAEL, uncertainty factor and DNEL for the four phthalates

mg/kg bw/day	NOAEL	LOAEL	Uncertainty factor	DNEL, external dose	DNEL, internal dose a	Notes on key studies	Endpoint and species	Commentary	Reference
DEHP	3	10	2.5*4*10 = 100	0.03	0.015	NOAEL 3 is close to NOAEL 5 from Wolfe and Layton	↓ AGD, ↑ Nipple retention, rat		Christiansen et al., 2010
	4.8	14	2.5*4*10 = 100	0.05	0.025#	NOAEL 5 is the highest NOAEL below the lowest LOAEL.Accepted in EU RAR and EFSA	Reproduction (germ cell depletion, ↓ testis weight), developmental toxicity, rat	EU RAR 2008, EFSA	Wolfe & Layton, 2003
	100	300	2.5*4*10 = 100	1	0.5		↓ Testosterone GD 18, rat		Howdeshell et al., 2008
	100	300	2.5*4*10 = 100	1	0.5		↓ Testosterone GD 18, rat		Hannas et al., 2011
	1.2	5	2.5*4*10 = 100	0.012	0.006		Reproduction (↓ daily sperm production, ↑ cryptorchidism)		Andrade et al., 2006
DBP		2	2.5*4*10*3 = 300 (EFSA use an assessment factor of 200)	0.0067 (EFSA 0.01)	0.0067# (EFSA 0.01)	Lowest LOAEL but far from other studies. Accepted in EFSA opinion 2005.	Reduced spermatocyte development PND 21, mammary gland changes in adult males, rat.	EFSA 2005	Lee et al., 2004
	200	1000	2.5*4*10 = 100	2	2	Same study as above	↓ AGD ↑ nipples, rat		Lee et al., 2004
	-	250 (330)	2.5*4*10*3 = 300	0.8 (1.1)	0.8 (1.1)		↓ AGD, rat		Ema & Miyawaki, 2001
	250	500	2.5*4*10 = 100	2.5	2.5		↓ AGD, rat		Jiang et al., 2007
	50	250	2.5*4*10 = 100	0.5	0.5	Alternative NOAEL 50	↓ AGD, rat		Zhang et al., 2004
	-	250	2.5*4*10 *3= 300	0.8	0.8		Testis effect, rat		Gray et al., 1999
	-	100	2.5*4*10*3 = 300	0.3	0.3		At 100: delayed preputial separation. At 250: ↓ AGD ↑ nipples, rat.	EFSA 2005, EU RAR 2003	Mylchreest et al., 1999
	50	100	2.5*4*10 = 100	0.5	0.5	Alternative NOAEL 50	↑ nipples, rat		Mylchreest et al., 2000
	10	50	2.5*4*10 = 100	0.1	0.1		↓ Testosterone GD 19, rat		Lehmann et al., 2004

mg/kg bw/day	NOAEL	LOAEL	Uncertainty factor	DNEL, external dose	DNEL, internal dose a	Notes on key studies	Endpoint and species	Commentary	Reference
	100	300	2.5*4*10 = 100	1	1		↓ Testosterone GD 18, rat		Howdeshell et al., 2008
	-	52	2.5*4*10*3 = 300	0.2	0.2		Embryotoxicity, rat. ↓ No of live pups, pup wt. Organ wt change from 520	EU RAR 2003	Wine et al., 1997
DiBP	125	250	2.5*4*10 = 100	1.25	1.25#	125 is highest NOAEL and close to NOAEL 100 from studies on testosterone	↓ AGD, ↑ Nipple retention, rat		Sallenfait et al., 2008
	100	300	2.5*4*10 = 100	1	1		↓ Testosterone GD 18, rat		Howdeshell et al., 2008
	100	300	2.5*4*10 = 100	1	1		↓ Testosterone GD 18, rat		Hannas et al., 2011
BBP	50	250	2.5*4*10 = 100	0.5	0.5#	50 – Highest NOAEL	↓ AGD, rat (nipples at higher doses)	EFSA, EU RAR 2007	Tyl et al., 2004
	100	500	2.5*4*10 = 100	1	1		↓ AGD, repro organs, rat	EU RAR 2007	Nagao et al., 2000
	-	100	2.5*4*10 = 300	0.3	0.3		↓ AGD, testicular effects		Aso et al., 2005
	100	300	2.5*4*10 = 100	1	1		↓ Testosterone GD 18, rat		Howdeshell et al., 2008
	185	375	2.5*4*10 = 100	1.8	1.8		Developmental toxicity, rat	NTP 2003	Ema et al., 1990
	182	910	2.5*7*10 = 175	1	1		Developmental toxicity, mice	NTP 2003	Price et al., 1990 in NTP-CEPHR, 2003

Uncertainty factor: 2.5 interspecies; allometric scaling: 4 for rats, 7 for mice; 10 intraspecies; 3 from NOAEL to LOAEL

[a] oral absorption fraction=0.5 for DEHP and 1 for other compounds see Table 15. [#] Selected DNEL, based on lowest NOAEL and studies accepted in EU RAR or by EFSA opinions.

In the EU risk assessment reports for these phthalates (DEHP, DBP and BBP), the reproductive and developmental effects are considered critical, i.e. are seen at the lowest dose levels, and Table 17 compares NOAELs selected here to overall NOAELs concluded in EU Risk assessment reports.

Table 17 Selected NOAELs compared with NOAELs from EU risk assessment reports (EU RAR). No EU Risk assessment report is available for DIBP.

mg/kg bw/day	DNEL, internal dose (from Table 16)	NOAEL for DNEL selection in this document	Endpoint for NOAEL selection in this document	Overall NOAEL from EU RAR	Endpoint for NOAEL selection in EU RAR
DEHP	0.025	**4.8**	Testis toxicity, developmental toxicity	4.8	Testis toxicity, developmental toxicity
DBP	0.0067	**2 (LOAEL)**	Reduced spermatocyte development PND 21, mammary gland changes (vacuolar degeneration and alveolar atrophy) in adult male offspring	52 (LOAEL)	Embryotoxicity
DIBP	1.25	**125**	↓ AGD, ↑ Nipple retention	-	-
BBP	0.5	**50**	↓ AGD, rat	50/100	↓ AGD, reproductive organ weight

Table 17 shows that the largest difference between NOAEL selection in the EU RAR and in this report is seen for DBP. DBP appears to be a more potent anti-androgen than DEHP, but the DNEL for DBP is based on a study on male mammary gland changes during development and in adulthood, and it has not been possible to find any literature on the effects of developmental phthalate exposure on male mammary glands for other phthalates. Thus, NOAELs/LOAELs for mammary gland effects have not been determined for any of the other phthalates listed in Table 16. It can not be excluded that if this effect was studied on the other phthalates DNEL's for the other phthalates could have been lower. Further, it should be noted that the study by Lee et al., 2004, was not included in the EU RAR for DBP from 2003, but the study is accepted in an EFSA opinion from 2005.

B 5.9.1 Epidemiological studies

The four phthalates DEHP, DBP, DIBP and BBP are all classified as toxic to reproduction in category 1B. These four phthalates are considered to have endocrine disrupting effects with similar mode of action.

The endocrine disrupting effects that are suspected to be relevant in humans in relation to the four phthalates are congenital malformations of the male reproductive organs, reduced semen quality, reduced male reproductive hormone levels, and changes in pubertal timing including changes in breast development. Based on the current knowledge of the biology of testicular cancer and breast cancer as well as of the shared risk factors of these cancers and some of the abovementioned effects it has also been speculated whether prenatal exposure to phthalates may play a role in the increasing incidence levels of these two hormone dependent cancers.

<u>Regarding congenital malformations of the male genitalia</u>

Although prenatal exposure to DBP and DEHP in relatively high doses in rat studies results in cryptorchidism and hypospadias no direct link between pre- and perinatal phthalate exposure and these malformations of the male genitalia has been proven in humans. There are, however, some circumstantial findings that indicate that phthalate exposure could play a role. Cryptorchidism and hypospadias are both conditions caused by insufficient testosterone action during respectively descent of the testes and the formation of the penis. In a case-control study of phthalate levels in breast milk samples (n=130) from mothers of cryptorchid and healthy boys (62 cryptorchid/68 healthy boys) from a large prospective Danish-Finnish mother-child cohort no association between phthalate levels and cryptorchidism could be seen. However, an association between phthalate level in maternal milk and the male reproductive hormone profiles in the male infants was observed, indicating that testicular function of the more exposed boys were affected (phthalates in breast milk and changed hormone levels in 3 months old Infants – Main KM et al, Environ Health Perspect. 114(2): 270-276, 2006). The findings are in line with another recent human study showing decreased virilization in infant boys exposed to phthalates prenatally. The perinatal exposure of the boys was based on maternal urinary phthalate levels during midpregnancy and the effects were manifested as a significantly decreased anogenital distance (AGD) in the most exposed boys compared to the least exposed boys. The AGD is determined by testosterone action during fetal development (Swan et al Environ Health Perspect, 113(8): 1056-61, 2005). Also in this study, no significant direct association between prenatal exposure to phthalates and cryptorchidism was found. Still, however, the boys with shorter AGD were more likely to be cryptorchid than the boys with longer AGD.

Regarding semen quality

Large internationally coordinated studies on semen quality of men from the general population in different European countries including France, Finland, Scotland, Estonia and Denmark found large differences between the countries and especially in Denmark a large proportion of the men had semen quality in the sub-fertile range while Finnish men seem to have significantly better semen quality. Although genetic differences cannot entirely be ruled out as causes, the considerable differences indicate different exposures or lifestyle changes in the four populations. (Jørgensen et al, Human Reprod. 16 (5)1012-19 (2001), Jørgensen et al, Human Reprod. 17(8):2199-208, (2002)). However, in the aforementioned study on phthalate levels in breast milk from a Danish-Finnish mother-child cohort the levels of phthalate metabolites measured were actually higher in Finnish breast milk than in Danish, while other EDC levels including some dioxins, PCBs, hexachlorobenzene and dieldrin were significantly higher in the Danish samples. This seems to imply that Danes are not more exposed to phthalates than Finns and consequently that phthalate exposure is not likely to play a major role for the poorer semen quality of Danish men. However, although current exposures may have an effect on men's semen quality, a man's general potential for producing sperm is dependent on his testicular capacity, which in general is determined already during the fetal development of the testis. According to the TDS hypothesis fetal exposure may play an important role for the testicular function in adult life. Recently, this link between fetal development – and more specifically fetal testosterone activity – and sperm quality in adult life was further corroborated by a new study showing a significant positive correlation between a man's AGD and his semen quality (Mendiola et al, Environ Health Perspect, online 4 March 2011). We do not know the phthalate exposure in Denmark nor in Finland at the time when the men who delivered semen for the semen quality studies were born **20-35** years ago as our measurements of phthalates in Danish and Finnish breast milk were done on samples collected only 10-12 years ago. The latest studies in Finland seem to indicate that the semen quality of young Finnish men has

continued to decrease and now approach the levels seen in the Danish male population (Jørgensen et al, Int. J. Andrology 2011 (Epub)).

Regarding pubertal timing

A substantial decline in the age of onset of puberty in Danish girls was observed over the recent 15-year period (from 1991-93 to 2006-08) and manifested as earlier age at breast development (Aksglaede et al, Pediatrics (5): e932-e-939 (2009)). A study from Puerto Rico found that the phthalate concentration in samples from girls with premature thelarche (breast development) was significantly higher than in samples from age-matched controls (Colon et al, Environ Health Perspect, 108(9):895-900 (2000)). However, it was debated whether the data could be flawed by methodological problems as the phthalate levels measured was much higher than measured in e.g. the USA. Subsequent a small case-control study on girls with precocious puberty found not difference in urinary phthalate levels of healthy girls and girls with precocious puberty (Lomenick et al. Phthalate exposure and precocious puberty in females, J. Pediatr. 156:2215, 2010). The contradicting findings may lay in the differences in the inclusion criteria between the two studies. In the Puerto Rico study the inclusion as case was based on the presence of premature thelarche only, while in the USA study the inclusion criteria for cases was presence of central precocious puberty. Premature thelarche is sometimes seen isolated without activation of the pituitary-gonadal hormone axis. In this respect it is interesting that although a one year decrease in age at breast development was observed in the Danish study the girls did not have significantly different endogenous hormone levels compared to age-match girls studied 15 years earlier. Thus, it seems to be breast development but not the activation of the pituitary-gonadal hormone axis that seem to be advanced.
In rat studies prenatal phthalate exposure is associated with delayed – not advanced – puberty (estimated as the age of vaginal opening) in female offspring, while exposure of prepubertal rats has been shown to advance the age of vaginal opening (Ma et al. 2006 Toxicol. Sci. 93:164-71) so the effects of phthalate exposure on puberty development may depend on the timing of exposure. Humans are the only mammalian that has permanently protuberant breasts, even when not lactating and as such breast development during puberty cannot directly be compared with puberty in rats. However, in utero exposure to phthalates has been shown to induce modifications in the morphology and the gene expression profile of the mammary gland that persist postnatally and potentially may make the breast tissue more susceptible to subsequent exposures (Moral et al, Environ Health, 10:5, 2011).

Regarding testicular cancer

A significant increase in the incidence of testicular cancer has occurred over the last decades in many countries indicating that environmental or lifestyle factors play a role in testicular cancer. Unfortunately no animal model for testicular cancer exists, as no laboratory animal species seems to develop testicular cancer. However, testicular cancer are linked to other problems with male reproduction, and having cryptorchidism or poor semen quality are risk factors for developing testicular cancer indicating that these condition share aetiology. Thus, any exposure that is suspected to play a role in cryptorchidism or decreased testicular function may also be suspected to play a role in testicular cancer. Interestingly, Finnish men have not only experienced a recent adverse trend in semen quality as mentioned above; they are also experiencing a concurrent adverse trend in the incidence of testicular cancer (Jørgensen et al, Int. J. Andrology 2011 (Epub)).

As shown above epidemiological studies are generally associated with great uncertainties due to all kinds of exposures during the measured person's behaviour, background, smoker/non-smoker, diet,

weight etc. It is therefore diffult to draw exact conclusions on these studies, but they could be seen as contributing to the overall picture.

B 5.10 Other effects

Not relevant

B 5.11 Derivation of DNEL(s)/DMEL(s)

The DNELs are based on NOAELs for anti-androgenic effects in developmental studies, i.e. doses are administered to adult female rats during gestation and lactation. As such, risk calculations for pregnant women are relevant. Risk calculation for 2-year olds and 6/7-year olds are also based on these NOAELs and they may not be relevant for this age group. As sufficient dose-response studies in animal models mimicking direct exposure of children are lacking, DNELs based on NOAELs of dams are used for toddlers and children, but some uncertainty is associated with this DNEL. Due to the lack of relevant studies it is not possible to evaluate whether higher or lower NOAELs may be more relevant for toddlers and children. The prenatal and early postnatal period is most likely the most sensitive period for the effects of phthalates and this could point towards higher NOAELs for children than fetuses and newborns, i.e. that the selected NOAELs lead to an underestimation of DNELs and in turn an overestimation of the risk. However, the NOAELs in experimental studies are based on the dose levels given to the dams and are not the dose levels given directly to the fetuses and the newborn. The internal dose levels received by the newborn experimental animals via lactation are most likely lower than the dose levels given to the pregnant dams as only a fraction is likely to be transported across the placenta or excreted in maternal milk. This means that internal NOAELs of pups (neonatal and lactating) may actually be lower than internal NOAELs of the dams.

In some cases a NOAEL from a repeated dose study may be preferred for risk assessment in a child-specific scenario. However, in this case the anti-androgenic endpoints are selected as relevant for cumulative risk assessment of phthalates, and repeated dose studies would not be preferred as these studies do not include hormone-sensitive endpoints.

Overall, NOAELs based on anti-androgenic effects in developmental studies are considered the most relevant available data for the current risk assessment.

See also section B 5.9 Toxicity for reproduction.

B.6 Human health hazard assessment of physico-chemical properties

Not relevant

B.7 Environmental hazard assessment

Not relevant

B.8 PBT and vPvB assessment

Not relevant

B.9 Exposure assessment

B.9.1 General discussion on releases and exposure

B.9.1.1 Summary of the existing legal requirements

DEHP, DBP and BBP are restricted in toys and childcare articles in concentrations above 0.1 % according to REACH Regulation Annex XVII, entry 51 and 52.

The four phthalates are restricted in toys from July 2013 in concentrations above the specific classification limits (Directive 2009/48/EC on the safety of toys) and DEHP, DBP and BBP are furthermore included in Annex XIV of the REACH Regulation and are thus subject to the authorisation process. However, the authorisation process does not cover placing on the market of articles containing the phthalates and therefore not any imported articles. Numerous articles could therefore still contain the four phthalates. DIBP is on the candidate list to be included on the Annex XIV and are now on the 2nd recommendation by ECHA for this inclusion. All four phthalates are thus on the candidate list with the obligations for industry to provide information downstream (including consumers) of the use in articles if the content is higher than 0.1%.

Electrical and electronic products are regulated by the RoHS Directive, and DEHP, BBP and DBP are subject to - as part of the first-coming review - to be addressed in order to investigate the necessity to include these substances in the Directive.

As far as food packaging is concerned, the use of DEHP in food contact materials is already restricted under Commission Directive 2007/19/EC of 30 March 2007 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. DEHP can be used in non fatty food contact materials for repeated use which includes such items as tubes and conveyer belts, provided the migration of the plasticiser does not exceed the Substance Migration Limit (SML) of 1.5 mg/kg food.

B.9.1.2 Summary of the effectiveness of the implemented operational conditions and risk management measures

Phthalates are found primarily in PVC as softeners but can also be found in other plastics in low concentrations. Phthalates can also be found in e.g. dispersions, paints and varnishes, as emulsifiers, repellents and carrier fluids in biocides, in cosmetics etc.

Several reports indicate that single articles (containing very high concentrations of phthalates) e.g. single types of plastic sandals (Danish EPA, 2010c) and single erasers (Danish EPA, 2007) will contribute to a very high exposure to one or more of the four phthalates. Individuals are exposed to these phthalates through inhalation (phthalates emitted from wall paper, floor covering and other sources), ingestion (via e.g. food, toddlers suckling on plastic materials), and dermal exposure for their whole lifetime since the intrauterine life.

As shown in the rest of this section the exposure to these four phthalates raises concern with risk characterisation ratios (RCRs) well above 1 when considering the total exposure from all relevant articles to the four phthalates (the RCR for a chemical is the ratio between exposure level and DNEL).

This implies that the existing and already implemented operational conditions and risk management measures are too limited as they do not take into consideration the widespread occurrence of the four phthalates in numerous articles not covered by existing legislation. Furthermore the documentation presented in this dossier implies that the total exposure from indoor environment, food and articles could pose a risk.

Even though a high exposure to the four phthalates may be due to exposure to the phthalates from single articles, it is essential to include all other articles contributing to the concentration of phthalates in the indoor environment. In this respect none of the regulatory measures taken so far are sufficient to eliminate the exposure to the four phthalates.

B.9.2 Manufacturing

An estimation of the contribution to the total emissions of DEHP from different life-cycle stages is given in the RAR for DEHP:

- Production of DEHP ≈ 2.5%
- Industrial uses ≈ 2.5%
- End-product uses ≈ 32%
- Waste handling* ≈ 63%

It is evident that the main sources are the articles themselves and waste handling of the articles and not the production of the substance.

Workers
The exposure scenarios considered for workers in the EU risk assessment concern exposure to DEHP from production of DEHP, industrial use of DEHP and industrial end-use of preparations or materials containing DEHP (EU RAR, 2008a).

The conclusion is as follows:
"For the scenarios on production and industrial use, monitored data for inhalation exposure and modelled values for dermal exposure have been used as a realistic worst case. For the scenario industrial end-use of products containing DEHP, it is assumed that relatively high work temperatures, aerosol generation and considerable skin contact occur. There is not enough quantitative and qualitative information available on technical control measures and personal protective equipment used during production and processing to establish their effectiveness. 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 during production, processing and industrial end-use of preparations or materials containing DEHP. There is no concern for the acute toxicity, irritation and sensitising effects, carcinogenicity, and mutagenicity."

This shows that every additional exposure from any article whether it is at the production facilities or other places (like their homes) to the workers is contributing to the overall exposure of the workers and thereby contributing to the risk.

B.9.3 Human exposure

B.9.3.1 General information

In order to identify the most efficient risk reduction measures, the exposure from the different sources of the four phthalates DEHP, DBP, BBP and DIBP have to be identified.

Phthalates are found in high concentrations as softeners in PVC. Phthalates are also used in other plastics, in dispersions, paints and varnishes, as emulsifiers, in cosmetics and perfumes, and for other common applications.

The main sources of exposure to the four phthalates that are delt with in this proposal are articles, food and indoor environment. The exposure routes are via inhalation, ingestion and dermal and mucous contact.

The exposure to phthalates in articles may be due to direct contact between the article and the skin or mucous membrane, or oral, due to toddlers mouthing the article. The dermal and oral exposures are calculated based on analytical data, where the migration of the phthalates is measured to either artificial sweat or saliva. These data are reported in surveys from the Danish EPA (2010a-c).

The exposure from food is calculated based on data from the literature. Unfortunately only rather old data (mainly 2000 – 2009) on this can be found, and it can be expected that the level of phthalates in food would be lower today, because one of the major source of phthalates in the food was considered to be from food contact materials, which are regulated today. Even though, it can still be expected that food will contain phthalates, which is due to the fact that one of the sources to phthalates in food is the environment. Due to the lack of more specific data on the contribution from the environmental compartments, the mentioned literature data are used to calculate the exposure from food. There are no data that show that the exposure from food is significantly lower today compared to the exposure calculated based on the older data. There are limited data on the migration of phthalate from plasticized PVC into environmental media. However, due the total volume of plasticized PVC produced, it is possible that PVC or other polymer/polymericlike materials containing phthalates may be long-term and dispersive sources of human and environmental exposures to phthalates (US EPA 2009).

Furthermore, the indoor environment acts as another source contributing to the total exposure to phthalates. Phthalates are found in the air (gas phase and particles in air) and the dust in the indoor environment contributing to the human intake of phthatales. The exposure levels of phthalates from the indoor environment are found in the literature for the levels in dust, and by calculations and modelling for the levels in indoor air (gas phase and particles in air). The phthalates found in dust, particles in air in the indoor environment, as well as the phthalates found in articles (within a

building/ indoor environement) will emit over time, and thereby contribute to the phthalate concentration in the indoor air.

There are other exposures to phtalates which are not delt with in this proposal, as for example the exposure from medical devices and medicine. The use of these kinds of articles may be nessecary for health reasons and other considerations have to be taken into account.

As the exposure is expected to differ within different age groups, it is decided to divide the population in three age groups, 2-year olds, 6/7-year olds and adults. The reasoning for this is described in section A. Proposal. The exposure can even be expected to differ within these groups, due to different behaviour or stage of development. In order to show these differences the exposure is extimated for:
- the 95 percentile,
- the highest reported median values and
- the lowest reported median values.

This was possible for the exposure calculation of dust and food, as these calculations were based on literature data. The lowest median and the highest median are are based on a realistic scenario, which is identical for the calculations of exposure from articles and indoor air. The exposure calculated for the 95 percentile is based on a realistic worst case scenario. The assumptions for the realistic and the realistic worst case scenarios are explained in the section about exposure from articles (***B.9.3.2.2 Exposure from articles***).

B.9.3.2 Exposure estimation

The following sections present a literature search on best available exposure estimates on human exposure to the four phthalates DEHP, DBP, BBP and DIBP via articles, food and indoor environment and furthermore on biomonitoring studies (phthalates measured in urine samples from human populations). Furthermore, assessments of exposure from selected articles are based on analyses on content and migration of the four phthalates. Exposure data from indoor air are based on calculations and simulations performed on the background of measured concentrations of phthalates in selected articles.

These estimates are converted to internal dose estimates (µg/kg bw/day) when the absorption is less than 100 %, and "realistic worst case" exposure estimates are suggested. Exposure estimates are calculated for 2-year olds (15.2 kg bw), 6/7-year old (23.1 kg bw) and for adults (60 kg bw).

The weight for 2-year olds of 15.2 kg is the mean body weight of children just turned 3 years old in Denmark, whereas children just turned 2 years weigh in average 13 kg (Andersen, 1982). In this report, a body weight of 15.2 kg is used, as this corresponds to the body weight used in the project from the Danish EPA on chemical exposure of 2-year olds (Danish EPA, 2009). In some cases, data for children older than 7 years of age are used as exposure estimates for the group of 6/7-year olds. For adults, a body weight of 60 kg is used as a realistic weight of a woman in the reproductive age, i.e. the subpopulation that may need protection from reproductive toxicants (ECHA, 2010).

B.9.3.2.1 Occupational exposure

Workers can be exposed to the four phthalates during manufacturing of articles – not only due to direct "hands on" contact, but also due to the emissions from e.g. industrial extrusion processes or the presence of articles like e.g. PVC flooring at the production site. Other occupational exposures can come from different job situations in private households, nurseries, offices, hospitals, kinder gardens etc. Examples are the emissions to the indoor air from floorings, wallpaper, curtains, carpet tiles, tablecloths and curtains etc. In this regard the same calculations and reasoning given for consumers can be used for the estimation of occupational exposure though considering the special protection prescribed in the regulations limiting the occupational exposure with specific instructions, use of PPE´s, established local exhaust and ventilation, etc.

B.9.3.2.2 Exposure from articles

This exposure assessment is performed based on selected articles from which a high exposure of the four phthalates is expected. Exposure to the four phthalates will also occur from other articles. The main focus in this proposal is directed to articles to which children have a direct contact, as well as to articles, where adults have a long and direct contact. This will include for example plastic sandals, bags for toys, erasers etc. Articles can also contribute to the exposure to phthalates from dust and air (gas phase and particles in air). The exposure from dust and air is calculated in section B.9.3.2.3 Exposure from indoor environment.

The use of the articles could vary between the 2-year olds and 6/7-year olds, as it is expected that children under the age of 3 will mouth articles to a larger extent than other age groups. Other articles like for example training balls and sex toys are considered as articles that adults will be exposed to primary via dermal (mucous membrane) contact.

Exposure scenarios in relation to articles are mainly based on the three surveys performed in 2010 (Danish EPA, 2010a; Danish EPA, 2010b; Danish EPA, 2010c) of a broad variety of plastic articles containing phthalates placed on the Danish market. The articles chosen for these studies are seen as representative for other articles found in the indoor environment or articles with direct skin contact as it is impossible to measure all article (groups) where the four phthalates can be found. Besides, the phthalates have exactly the same function and use in the plastic so it is relevant to consider some articles as representing other article groups. Alongside these new studies two surveys from the Danish EPA from 2006 (Nilsson et al., 2006) and 2007 (Svendsen et al., 2007), where relevant articles have been found to contain one or more of the four phthalates (school supplies, sandals and sex toys) have been used in the calculations below..

The exposure to phthalates from articles is calculated as dermal or oral exposure.

B.9.3.2.2.1 Dermal exposure

The dermal exposure is calculated based on the following equation from the ECHA guidance on safety assessment, chapter R15, from 2010 (ECHA, 2010).

$$D_{der} = \frac{Q_{prod} \cdot Fc_{prod} \cdot Fc_{migr} \cdot F_{abs} \cdot F_{contact} \cdot T_{contact}}{BW} \cdot n$$

The product $Fc_{prod} \bullet Fc_{migr}$ corresponds directly to the results from the migration analyses.

where

D_{der}	Daily dermal dose (amount of chemical compound taken up)	µg/kg BW/day
Q_{prod}	Amount of product used	G
Fc_{prod}	Weight fraction of the compound in the product (decimal fraction between 0 and 1)	
Fc_{migr}	Fraction of compound that migrates out of the product per unit time	µg/g per hour
F_{abs}	Fraction of the applied compound that is absorbed through the skin (decimal fraction between 0 and 1)	
$F_{contact}$	Fraction of the contact area (to account for the fact that the product is only in partial contact with the skin)	M^2/m^2
$T_{contact}$	Duration of exposure per event	Hours
N	Number of exposures (events)	per day
BW	Body weight (BW)	kg

Exposure durations are given as a best, realistic estimate for the three age groups. Body surface area to be exposed is calculated based on ECHA guidance on information requirements and chemical safety assessment Chapter R.15: Consumer exposure estimation version 2, April 2010 and US EPA Child-Specific Exposure Factors Handbook 2002.

In Table 18 and Table 19 article and age specific exposure estimates are given. The assumptions made on exposure duration and body surface areas are made as realistic worst case scenarios.

Table 18 Estimated dermal exposure durations and exposed body surface area for the different consumer product groups analysed (Danish EPA, 2010b; Danish EPA 2010c).

Data	Children 2-year olds	Children 6/7-year olds	Adults
Weight kg	15.2	23.1 [1]	60
Dermal exposure duration plastic sandals(hours/day)	10	12	16
Shoe size	26	32	44*
Exposed surface area (cm^2) two feet	170	228	444
Dermal exposure duration bags (min/day)	60	60	60
Exposed surface area bags (cm^2)	87	121	183

Data	Children 2-year olds	Children 6/7-year olds	Adults
Dermal exposure duration shower curtains (min/day)	10	10	0
Exposed surface area shower curtains (cm^2)	86	121	0
Dermal exposure duration oilcloth and dinner mats (min/day)	60	60	60
Exposed surface area oilcloth and dinner mats (cm^2)	366	580	861
Dermal exposure duration swimming equipment (min/day)	60	60	0
Exposed surface area swimming equipment (cm^2)	385	674	0
Dermal exposure duration swimming pools (min/day)	30	30	0
Exposed surface area swimming pools (cm^2)	2015	3203	0
Dermal exposure duration balance balls (min/day)	10	10	10
Exposed surface area balance balls (cm^2)	551	940	1227
Dermal exposure duration training balls (min/day)	10	10	10
Exposed surface area training balls (cm^2)	173	242	366
Dermal exposure duration sex toys (min/day)**	0	0	10
Exposed surface area sex toys (cm^2)**	0	0	120

*Adult shoe size 44 seem not to be in proportion with the bodyweight of 60 kg. A size 44 is chosen as a worst case. It is also accepted to use a hand size of a man but a bodyweight of 60 kg (ECHA Guidance R15, 2010)
**From Nilsson et al., 2006

Table 19 Assumptions made to calculate the exposed surface area.

Data	**Children 2-year-olds****	**Children 6/7-year-olds****	**Adults***
Shoes	90 % of measured sole area on 95 cm^2 for one foot	90 % of measured sole area on 127 cm^2 for one foot	90 % of measured sole area on 244 cm^2 for one foot
Bags	One palm corresponding to ¼ of the surface of two hands	One palm corresponding to ¼ of the surface of two hands	One palm corresponding to ¼ of the surface of two hands
Shower curtains	One palm corresponding to ¼ of the surface of two hands	One palm corresponding to ¼ of the surface of two hands	No exposure
Oilcloth and dinner mats	Two palms and two forearms corresponding to ½ of the surface of two hands and ¼ of the surface of two arms	Two palms and two forearms corresponding to ½ of the surface of two hands and ¼ of the surface of two arms	Two palms and two forearms corresponding to ½ of the surface of two hands and ¼ of the surface of two arms
Water wing	Two upperarms corresponding to ½ of the surface area of one arm	Two upperarms corresponding to ½ of the surface area of one arm	No exposure

Data	Children 2-year-olds**	Children 6/7-year-olds**	Adults*
Swimming pools	Back and back of the legs corresponding to ½ of the surface of the trunk and ½ of the surface of the legs	Back and back of the legs corresponding to ½ of the surface of the trunk and ½ of the surface of the legs	No exposure
Balance ball (65 cm in diameter)	Two palms and two front of thighs corresponding to ½ of the surface of two hands and ¼ of the surface of two legs	Two palms and two front of thighs corresponding to ½ of the surface of two hands and ¼ of the surface of two legs	Two palms and two back of the legs.
Training ball (25 cm in diameter)	Two palms corresponding to ½ of the surface of two hands	Two palms corresponding to ½ of the surface of two hands	Two palms corresponding to ½ of the surface of two hands

*Surface areas from ECHA guidance on information requirements and chemical safety assessment, Chapter R.15: Consumer exposure estimation.

** Surface areas from US EPA Child-Specific Exposure Factors Handbook 2002:

Total surface area of 2 years old is 6530 cm^2 (95 percentile US EPA, 2002)
Hands are 5,3 % of total body surface area (US EPA, 2002)
Arms are 11,80 % of total body surface area (US EPA, 2002)
Legs are 23,20 % of total body surface area (US EPA, 2002)
Trunk are 38,5 % of total body surface area (US EPA, 2002)

Total surface area of 6/7 years old is 10300 cm2 (95 percentile US EPA, 2002)
Hands are 4,71 % of total body surface area (US EPA, 2002)
Arms are 13,10 % of total body surface area (US EPA, 2002)
Legs are 27,10 % of total body surface area (US EPA, 2002)
Trunk are 35,1 % of total body surface area (US EPA, 2002)

The dermal exposure is calculated based on the above assumptions and given in Table 19. Exposure is calculated based on the migration of phthalates from the given products. The detailed data and the conditions for the measured migration can be seen in Danish EPA, 2006a and Danish EPA, 2010a-c.

Table 20 Calculated dermal exposure of DEHP, DIBP and DBP in consumer products based on migration analysis and exposure assumptions.

Product	Measured migration of DEHP in µg/cm²/hour	Exposure in ug/kg bw/day DEHP			Measured migration of DIBP in µg/cm²/hour	Exposure in ug/kg bw/day DIBP			Measured migration of DBP in µg/cm²/hour	Exposure in ug/kg bw/day DBP		
		Children 2-year-olds	Children 6/7-year-olds	Adults		Children 2-year-olds	Children 6/7-year-olds	Adults		Children 2-year-olds	Children 6/7-year-olds	Adults
Plastic sandals median exposure*	0.42 (2-year) 1.02 (6/7-year) 0.73 (adults) ***	0.8986	1.8700	1.1664	42.65 (adults) ***	0	0	4.9212	0.25 (adults) ***	0	0	0.8629
Plastic sandals worst case exposure**	2.29 (2-year) 0.65 (adults) ***	3.6179	0	1.4332	49.43 (2-year) 13.47 (adults) ***	3.5578	0	2.6122	51.25 (6/7-year) 28.35 (adults) ***	0	3.9076	5.4971
Bag	0.21	0.0598	0.0551	0.03203	0	0	0	0	0	0	0	0
Shower curtain	0.08	0.0038	0.0035	0	0	0	0	0	0	0	0	0
Oilcloth	0.09	0.1083	0.1130	0.0646	0	0	0	0	0	0	0	0
Water wing	0.08	0.1014	0.1168	0	0	0	0	0	0	0	0	0
Swimming pool	0.11	0.3645	0.3813	0	0	0	0	0	0	0	0	0
Balance ball	0.38	0.1150	0.1289	0.0648	0	0	0	0	0	0	0	0
Training ball	0	0	0	0	5,8	1.1005	1.0151	0.5897	0	0	0	0
Sex toy****	0.06/55	0	0	0.02/18.3	0	0	0	0	0	0	0	0

*Median exposure is under dynamic extraction and sunscreen applied on the strap of the sandal.

**Worst case exposure is the highest calculated exposure under dynamic extraction and sunscreen applied on the strap of the sandal.

***The migration is measured from three different sandals depending on age and therefore given in brackets in µg/kg bw/day after the exposure calculation.

****The first value is based on the migration to artificial sweat and the second value is based on the migration to artificial sweat + oil based lubricant (worst case scenario).

A realistic worst case scenario for sandals is chosen where the migration from the sole is measured to artificial sweat under dynamic conditions on a shaking table and the migration from the strap is measured to sunscreen under dynamic conditions. This case is chosen as it does not seem realistic that sunscreen will be applied to the sole of the foot, but it is realistic to apply sunscreen to the upper side of the foot. In the risk characterization there are refered to "lowest median" and "highest median". The figures used for the calculation of the risk for plastic sandals are the median exposure and the highest calculated exposure, respectively.

Analysis of the migration of phthalates to sunscreen under dynamic conditions showed that the migration of DEHP increased by a factor 149.5 and the migration of DBP and DIBP increased by a factor 3.4. Using dynamic conditions, without the use of sunscreen, gave an increase in the migration by a factor 25.1 for DEHP and 1.1 for DBP and DIBP (Danish EPA, 2010c). In the table above these factors are applied to the calculated exposure of phthalates from straps on the shoe.

Example of the calculation of the dermal exposure to DEHP from the bag for a 2-year-old:

$$D_{dermal} = \frac{0.21\mu g/cm^2 \times 86.52 cm^2 \times 0.05 \times \left(\frac{60\,\text{min}}{60\,\text{min}}\right)}{15.2 kg} = 0.0598 \mu g/kgbw/day$$

Based on the following parameters:

Migration of DEHP	0.21 µg/cm^2/hour
Dermal absorption of DEHP	5 %
Exposed surface area	87 cm^2
Exposure time	60 min
Body weight	15.2 kg

The calculated dermal exposure estimates for all age groups (Table 20) are used to calculate the risk characterisation in section *B.10.1.5.1 Articles*.

B.9.3.2.2.2 Oral exposure

The oral exposure is calculated based on the following equation from the ECHA guidance on safety assessment, chapter R15, from 2010 (ECHA, 2010).

$$D_{oral} = \frac{Q_{prod} \cdot Fc_{prod} \cdot Fc_{migr} \cdot F_{oral\ contact} \cdot T_{contact}}{BW} \cdot n$$

The product $Fc_{prod} \bullet Fc_{migr}$ corresponds directly to the results from the migration analyses.

where

D_{oral}	Oral consumption daily dose	µg/kg BW/day

Q_{prod}	Weight of product one is exposed to	G
Fc_{prod}	Weight fraction of the compound in the product (decimal fraction between 0 and 1)	
Fc_{migr}	Fraction of compound that migrates per unit time	µg/g per hour
$F_{oral\ contact}$	Fraction of the contact area (to account for the fact that the product is only inside the mouth)	m^2/m^2
$T_{contact}$	Duration of exposure per event	Hours
N	Number of exposures (events)	per day
BW	Body weight (BW)	Kg

It is assumed that a 2-year old will mouth 10 cm^2 of the any consumer product. The assumptions made on exposure duration are made as realistic worst case scenarios and given in Table 21.

Table 21 Assumptions on oral exposure duration (Danish EPA, 2010b)

Data	Children 2-year-olds	Children 6/7-year-olds	Adults
Weight kg	15.2	23.1	60
Oral exposure duration bags (min/day)	10	0	0
Oral exposure duration shower curtains (min/day)	10	0	0
Oral exposure duration oilcloth and dinner mats (min/day)	10	0	0
Oral exposure duration swimming pools (min/day)	30	0	0
Oral exposure duration balance balls (min/day)	10	0	0
Oral exposure duration training balls (min/day)	10	0	0
Oral exposure duration erasers (min/day)	0	60	0

The oral exposure from articles are calculated and given in Table 22. The exposure is calculated based on the measured migration to artificial saliva. It is assumed that adults will not be exposed to phthalates from any of the articles via oral exposure. No migration of DBP and BBP were measured from the articles. The detailed data and the conditions for the measured migration can be seen in Danish EPA, 2007 and Danish EPA, 2010b.

Table 22 Calculated oral exposure of DEHP and DIBP from articles

Product	Measured migration of DEHP in µg/cm²/hour	Exposure in ug/kg bw/day DEHP			Measured migration of DIBP in µg/cm²/hour	Exposure in ug/kg bw/day DIBP		
		Children 2-year-olds	Children 6/7-year-olds	Adults		Children 2-year-olds	Children 6/7-year-olds	Adults
Bag	0.08	0.0088	0	0	0	0	0	0
Shower curtain	0.06	0.0066	0	0	0	0	0	0
Oilcloth and dinner	0.07	0.0077	0	0	0	0	0	0
Swimming pool	0.08	0.0088	0	0	0	0	0	0
Balance ball	0.24	0.0263	0	0	0	0	0	0
Training ball	0	0	0	0	3.7	0.4057	0	0
Eraser mouthing*	0.0833**	0	15.8	0	0	0	0	0
Eraser eating 8 mg*	440000***	0	176.0	0	0	0	0	0

*calculations of erasers are based on a child weighing 20 kg

**the migration in mg/g is divided with a factor of 6 to take into account that the migration was measured from small pieces of eraser, given a larger surface, and to take account of the uncertainty of the analysis which is 50 % divided with 2. It is assumed that the weight of eraser mouthed is 3.79 g corresponding to 1 cm of the eraser is mouthed.

***content in mg/kg

The calculated oral exposure estimates for all the age groups (Table 22) are used to calculate the risk characterisation in section *B.10.1.5.1 Articles.*

B.9.3.2.2.3 Uncertainties on the measurements of exposure from articles

There will be uncertainties when the exposure from articles is calculated. Assumptions are made on for example the body weight and the time of dermal or oral contact with the articles. In this case the assumptions are based on a realistic worst case scenario, and this will overestimate the

exposure for some persons. It is for example assumed that the same adult person is using sex toys and the plastic sandal with the highest migration of phthalates, and that this person is using all the relevant articles in the worst case use. This will overestimate the risk from articles, especially in the worst case scenario. It could be expected that some persons use some articles as set up in the median exposure scenarios and other articles as set up in the worst case scenario, and the risk could be somewhere between these two scenarios.

However, it should be noted that the specific articles included in these exposure estimates represent only a few of numerous sources of phthalate exposure from articles. For that reason, the cumulative exposure may easily reach levels of serious concern and a need of further control and limitation of the exposure to phthalates, especially for children.

B.9.3.2.3 Exposure from indoor environment

Exposure of phthalates from indoor environment can happen through indoor air (gas phase and particles in air) or dust. The exposures from these two sources are estimated as the exposure from the indoor environment.

B.9.3.2.3.1 Exposure to phthalates via dust

Studies on exposure to DEHP, DBP, DIBP and BBP via dust are summarized in Table 23.

Calculations are based on measured phthalate levels in dust (in µg/g dust) and an estimated intake of 100 mg dust per day for children and 50 mg dust per day for adults (Oomen et al., 2008; ECHA, 2010). Exposure estimates are calculated as internal exposures using the oral absorption fractions listed in Table 15 (50 % for DEHP uptake in adults and 100 % for children, and 100 % for adults and children for other phthalates).

$$Exposure_{internal} = \frac{C_{phthalate} \times D_{oral} \times F_{oral}}{BW}$$

$Exposure_{internal}$	Internal exposure
C_{phth}	Phthalate concentration in dust
D_{oral}	Daily intake of dust
F_{oral}	Fraction of phthalate absorbed
BW	Bodyweight

The calculated values of exposure from dust are not unrealistically high, as the values for dust intake (50 and 100 mg for adults and children, respectively) are not worst case estimates, but are selected as conservative, but still realistic estimates (Oomen et al., 2008). As this report also works with "realistic worst case" scenarios, calculations of exposure estimates should include exposure to dust with high contents of phthalate as well as exposure to high amounts of dust. Thus, data for 95th percentile level of phthalates in dust provide the best estimates of "realistic worst case" exposure to phthalates via dust. A central study was published by Bornehag et al. (2005), who measured dust levels in children's rooms in Sweden in 2001-2002 and compared measured values to studies in Germany, Norway and Denmark. This study included the largest number of samples of the studies listed in Table 23, and this study found slightly higher median phthalate concentrations in dust

compared to the other listed studies. This study focused on levels in children's rooms and may therefore be considered particularly relevant for estimating phthalate exposure of both children and adults spending time in children's rooms. 95th percentile dust concentrations from the Bornehag study are therefore used for calculation of realistic worst case estimates, and median dust concentrations are used for realistic median estimates. 52 % of the houses had PVC flooring in the child's bedroom, and BBP and DEHP levels in dust were significantly higher (2- and 1.2 fold, respectively) in rooms with PVC flooring than in those without PVC.

DEHP: Data on DEHP levels in dust are comparable for the listed studies with only a 2-fold difference between the study with the lowest measured median value (340 µg/g dust) and the study with the highest measured median value (770 µg/g dust). The highest values are seen in the study by Bornehag et al. (2005), and this study is used for estimation of DEHP intake from dust (gray-shaded values in Table 23).

DBP and BBP: For DBP and BBP a slightly larger variation in dust levels are seen, but still there is less than a 10-fold difference between the highest and lowest measured median values. Again, the study by Bornehag et al. (2005), shows the highest measured values, and as these values correspond to some of the other listed studies, this study is used for estimation of DBP and BBP intake from dust (gray-shaded values in Table 23).

DIBP: Two studies included measurement of DIBP in house dust. In an American study, a median level of 1.9 µg/g dust was detected, and the study by Bornehag et al. (2005), showed a median DIBP level of 45 µg/g dust with a 95th percentile of 311 µg/g (Bornehag et al., 2005; Rudel et al., 2003).

Generally, median estimates of exposure are comparable between studies, except for DIBP, where larger differences between studies were observed and may reflect differences in phthalate sources in the indoor environments studied.

Xu et al. (2010), presented intake estimates of DEHP from vinyl flooring based on model calculations. They estimated a median intake of 38 µg/kg bw/day and a 95th percentile intake of 180 µg/kg bw/day in children, and exposure estimates for children were 2 to 10 times greater than for adults. These estimates exceed intake estimates based on biomonitoring data, and the predicted dust concentrations are 2 to 10 fold higher than measured dust levels presented in Table 21. They found that the primary route of exposure is via oral intake of dust, whereas inhalation and dermal absorption contributed little to the total exposure (Xu et al., 2010). As these data are model calculations and not based on actual measured dust levels, they are not included in Table 23.

Table 23 Intake estimates of phthalates from house dust. Gray-shaded values are selected for comparison of "realistic worst case" estimates in Table 30.

Dust concentration: µg/g dust. Intake: µg/kg bw/day					**Toddlers 15.2 kg bw**		**Children, 23.1 kg bw**		**Adults, 60 kg bw**	
Study	**Country**	**N**	**Median conc.**	**95-p**	**Median daily intake***	**95-p daily intake**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**
DEHP										
Pohner 1997 **	Germany	272	450	2000	2.96	13.16	1.95	8.66	0.19	0.83
Oie 1997 **	Norway	38	640		4.21		2.77		0.27	
Butte 2001 **	Germany	286	740	2600	4.87	17.11	3.20	11.26	0.31	1.08
Becker 2002 **	Germany	199	416	1190	2.74	7.83	1.80	5.15	0.17	0.50
Clausen 2003 **	Denmark	23	858	2595	5.64	17.07	3.71	11.23	0.36	1.08
Rudel et al., 2003 in **	USA	120	340	854	2.24	5.62	1.47	3.70	0.14	0.36
Kersten 2003 **	Germany	65	600	1600	3.95	10.53	2.60	6.93	0.25	0.67
Fromme et al., 2004	Germany	30	703	1540	4.63	10.13	3.04	6.67	0.29	0.64
Becker et al., 2004	Germany	252	515	1840	3.39	12.11	2.23	7.97	0.21	0.77
Bornehag 2005	Sweden	346	770	4069	5.07	26.77	3.33	17.61	0.32	1.70
Abb et al., 2009	Germany	30	604		3.97		2.61		0.25	
Personal communication UBA, 2011***	Germany	10	310	1680 (90-p)	2.04	11.05	1.39	9.0	0.13	0.7
DBP										
Oie 1997 **	Norway	38	100		0.66		0.43		0.08	
Butte 2001 **	Germany	286	49	240	0.32	1.58	0.21	1.04	0.04	0.20
Becker 2002 **	Germany	199	42	160	0.28	1.05	0.18	0.69	0.04	0.13
Rudel et al., 2003	USA	120	20	44	0.13	0.29	0.09	0.19	0.02	0.04
Kersten 2003 **	Germany	65	47	180	0.31	1.18	0.20	0.78	0.04	0.15
Fromme et al., 2004	Germany	30	56	130	0.37	0.86	0.24	0.56	0.05	0.11
Bornehag 2005	Sweden	346	150	568	0.99	3.74	0.65	2.46	0.13	0.47
Abb et al., 2009	Germany	30	87		0.57		0.38		0.07	

* Daily intake is based on an estimated intake of 0.1 g dust per day for toddlers (15.2 kg bw) and children (23.1 kg bw), and 0.05 g dust per day for adults (60 kg bw). Intakes are thus 5.2 times higher for children than adults and 7.9 times higher for toddlers than for adults. ** Bornehag et al., 2005. *** Measurements in 10 German homes of boys in the age 5 to 8 year. The median weight of the group was 20.75 kg.

Dust levels: µg/g dust. Intake: µg/kg bw/day					Toddlers 15.2 kg bw		Children 23.1 kg bw		Adults 60 kg bw	
Study	**Country**	**N**	**Median**	**95-p**	**Median daily intake***	**95-p daily intake**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**
DIBP										
Rudel et al., 2003	USA	120	1.9		0.013		0.008		0.002	
Bornehag 2005	Sweden	346	45	311	0.3	2.0	0.19	1.3	0.04	0.26
BBP										
Oie 1997 in **	Norway	38	110		0.72		0.48		0.09	
Butte 2001 in **	Germany	286	49	320	0.32	2.11	0.21	1.39	0.04	0.27
Becker 2002 in **	Germany	199	15	207	0.10	1.36	0.06	0.90	0.01	0.17
Rudel et al., 2003	USA	120	45	277	0.30	1.82	0.19	1.20	0.04	0.23
Kersten 2003 in **	Germany	65	19	230	0.13	1.51	0.08	1.00	0.02	0.19
Fromme et al., 2004	Germany	30	30	218	0.20	1.43	0.13	0.94	0.03	0.18
Bornehag 2005	Sweden	346	135	599	0.89	3.94	0.58	2.59	0.11	0.50
Abb et al., 2009	Germany	30	15		0.10		0.06		0.01	

* Daily intake is based on an estimated intake of 0.1 g dust per day for toddlers (15.2 kg bw) and children (23.1 kg bw), and 0.05 g dust per day for adults (60 kg bw). Intakes are thus 5.2 times higher for children than adults and 7.9 times higher for toddlers than for adults. ** Bornehag et al., 2005

B.9.3.2.3.2 Exposure to phthalates from indoor air

B.9.3.2.3.2.1 General information

When SVOCs like e.g. phthalates are introduced into an indoor environment via e.g. vinyl floorings, wall paper, furniture and other articles consisting totally or partly of PVC, there is a tendency for them to redistribute from their initial location to all indoor surfaces, like e.g. furniture, dust, particles in the air and even hair and skin, where they are sorbed to these surfaces (Weschler et al., 2008). The surfaces that are not primary sources of SVOCs, will then act as sinks (sorptive reservoirs) and sources for subsequent emission.

Normal house dust, originating from wear and tear of e.g. textiles, furniture, vinyl flooring and via outdoor air, acts as a major sink in the indoor environment. This is due to the fact that dust consists of a huge number of small particles with very big surfaces to which the SVOCs can easily sorb. It has been found that airborne particles increase the rate at which DEHP is transported between rooms by a factor 5 relative to gas-phase transport (Weschler et al., 2008).

Because phthalate plastizisers are not chemically bound to the polymer matrix of e.g. vinyl flooring, and emission from the articles to air or other media usually occurs during the entire product use phase (Clausen et al., 2010; Xu et al., 2010), phthalates are among the most abundant contaminants in indoor air environment. Phthalates can persist indoors for years after they are introduced, even after the primary source is removed (Weschler et al., 2008).

Clausen et al. (2010) found that when the air flow rate is increased about 7 times, the specific emission rate (SER) is increased almost 6 times, and the system maintains an almost constant bulk air concentration despite different air exchange rates. If the surface materials contain phthalates and the only mechanism of removal is normal ventilation, it is impossible to avoid the phthalates in indoor air, and the substances can persist indoors for thousands of hours to many years (Weschler et al., 2008). High-frequent cleaning of all indoor surfaces (incl. walls etc.) will possible reduce the concentration of phthalates in the air. In our simulation cleaning of surfaces is not included.

It is noteworthy that many SVOC´s that are currently abundant in indoor environments were virtually absent from these environments a half-century ago (Weschler et al., 2008).

B.9.3.2.3.2.2 Exposure estimation

In order to identify realistic indoor air levels of the phthalates DEHP, DIBP, DBP and BBP, three different approaches have been used and the phthalate levels have been compared. Firstly, the indoor air levels of the phthalates were simulated in two different scenarios of realistic rooms furnished with furniture/materials marketed in Denmark. Secondly, a calculation has been made by applying the method referred to in the EU Risk Assessment Report on DEHP (EU RAR, 2008). Thirdly, these levels have been compared to air levels found in the literature.

B.9.3.2.3.2.3 Simulation

Two scenarios have been simulated: one children's play room and one bathroom. These two scenarios are chosen in order to simulate two rooms with PVC flooring (also to be used as worst

case scenarios), which is more common in children's play rooms and in bathrooms than it is in e.g. a dining room. The simulation is based on data from chemical analysis of for example PVC flooring, wall paper and balance balls (Danish EPA, 2001 and Danish EPA 2010a). The data on concentration of phthalates in vinyl flooring and wall paper is based on tests performed in 2001 (designated OLD) and 2010 (designated NEW). The concentrations of DEHP, DIBP, DBP and BBP in the rooms, are estimated using the model described in Xu (Xu et al., 2009) See annex 1.

In Table 24, the "steady-state" levels in the air of the different phthalates related to the different indoor simulations are found. The number of days seen in the table is the time it takes from the placing of a material in a room until the steady-state level is reached. A steady state situation is e.g. reached when the rate of emission from an internal surface in room equalises the elimination due to the air exchange rate.

Table 24 "Steady-state" levels of the four phthalates in the gas-phase of indoor air.

Sources / Phthalate	**DEHP µg/m^3**	**days**	**DIBP µg/m^3**	**days**	**DBP µg/ m^3**	**days**	**BBP µg/ m^3**	**days**	**TOTAL µg/ m^3**
CHILDRENS PLAY ROOM Vinyl flooring, wall paper, air mattress, chair and balance ball **NEW** vinyl flooring and wall paper	0.16	150	7e-6	0	8.2e-11	1	3.2e-6	1	**0.16**
CHILDRENS PLAY ROOM Vinyl flooring, wall paper, air mattress, chair and balance ball **OLD** vinyl flooring and wall paper	0.81	150	1e-5	0	8.2e-11	1	4.4e-5	1	**0.81**
BATH ROOM Vinyl flooring, wall paper and shower curtain **NEW** vinyl flooring and wall paper	0.26	150	1e-5	0	1.5e-9	1	1.8e-6	1	**0.26**
BATH ROOM Vinyl flooring, wall paper and shower curtain **OLD** vinyl flooring and wall paper	0.8	150	6e-6	0	1.5e-9	1	2.5e-5	1	**0.8**

As can be seen from table 22 above the by far dominating concentration of phthalates in the air originates from DEHP alone. This is the case even though the vapour pressure of DEHP is lower than of the other three phthalates. The explanation to this domination of DEHP in air is most probably the high content of DEHP in the articles compared to the content of the other phthalates in the materials.

It should be stressed that the only difference between the OLD and the NEW scenarios is the concentration of phthalates in the vinyl flooring and in the wall paper. It may be concluded that the higher concentrations of phthalates in the air in the OLD scenarios are only caused by the the higher concentrations of phthalates in the OLD vinyl floorings and OLD wall papers. The items, with which the rooms are furnished, are the same in both the OLD and the NEW scenarios

The levels of DEHP are brought further to Table 26.

B.9.3.2.3.2.4 Calculation

A calculation has been made by applying the method developed in the EU Risk Assessment Report on DEHP (EU RAR, 2008). In the calculation described in EU RAR the only phthalate source in the room is DEHP emitted from the vinyl flooring and the wall paper covered with plastisizer. The result of the calculation (9.4 µg/m^3) is 10 fold higher than the concentrations found in the simulations and in the other references, and it exceeds 5.3 µg/m^3, which is the saturated vapour pressure of DEHP at 20° C. All these levels are brought further to Table 26.

B.9.3.2.3.2.5 Data found in the literature

Larsen et al. (2007) made the following review of measured indoor air and particle in air concentrations of DEHP.

Table 25 Mean and maximum concentrations of DEHP in air measured (mainly sum of gas phase and particle in air phase) studies from different countries (Larsen et al., 2007).

Table 3
Mean and maximum concentrations of DEHP in air measured in studies from different countries

Study	# Obs	Mean conc. (µg/m^3)	Max. conc. (µg/m^3)
Personal sampling PM2.5 + vapour (Adibi et al., 2003)			
Poland	30	0.43	1.1
USA	30	0.22	0.41
Denmark, stationary sampling in an office (Clausen and Wolkoff, 1997)	9	0.17	0.50
Denmark, stationary sampling in four offices, a day-care centre and a classroom (Clausen et al., 1999)	12	0.33	1.1
USA, stationary sampling in homes (Sheldon et al., 1992)			
Day	115	0.14	1.0
Night	113	0.10	0.33
USA, homes (Rudel et al., 2003)	101	0.11	1.0
Japan, homes (Otake et al., 2001)	6	0.08	0.23
Germany (Fromme et al., 2004), homes,	59	0.19	0.62
kindergarten	73	0.60	2.25
USA, homes + workplaces (Rudel et al., 2001)	4	0.06	0.11
Japan (Otake et al., 2004) homes	27	0.32	3.13

UBA in Germany has measured the concentration of DEHP in 10 homes of boys in the age 5 to 8 years. The median measured content in the air was 0.243 µg/m^3 and the mean concentration was 0.254 µg/m^3 and the max concentration was 0.460 µg/m^3 (Personal communication UBA, 2011). These measurements are in the same range as the ones reported in the literature shown in Table 25 above.

Taking the mean concentrations from table 23 above, adding them and dividing with 12 (number of concentrations listed in the table) this result in a non-weighted average concentration of 0.23 µg/m^3.

An average concentration of 1 µg/m^3 is the result when performing the same calculation for the max. concentrations from the table. These levels are brought further to table Table 26.

B.9.3.2.3.3 Discussion and conclusion

Table 24 below is summing up the concentrations found by simulations, calculations and literature findings. See Annex 1 to this dossier for an example of the calculations made.

Table 26 Summary – Concentrations in indoor air

Simulations (DEHP is the dominating contributor, gas phase only) µg/m^3		Calculation according to EU RAR 2008a (gas phase only) µg/m^3		Literature levels of DEHP (sum of gas phase and particles in air) in indoor air (including offices, kindergartens and workplaces) µg/m^3	
Children´s play room, new Children´s play room, old Bathroom, new Bathroom, old	0.16 0.81 0.26 0.8	Children´s play room DEHP saturated vapour pressure, 20° C	9.4 5.3	Larsen et al. (2007) max. Larsen et al. (2007) mean	1 0.23

As can be seen from the table above, the result of the EU-RAR calculation, applied to the simulated Childrens playroom scenario (9.4 µg/m^3) is 10 fold higher than the concentrations found in the simulations and in the literature (which also comprise particles in air), and twice the the level of DEHP saturated vapour pressure at 20° C. This may be due to the fact that the EU RAR calculation method is rough and based on very few data, amongst others a fixed emission rate of 3 x 10^{-4} ug/m^2/s. The emission rate depends on the concentration in the room air, the time, the sinks (sorptive reservoirs), the material concentration etc., and is not a constant. This is in opposition to the assumption made in the EU RAR method. In our case the initial value of the emission rate is approximately 0.0025 ug/m^2/s but it drops during emission, when the room air becomes saturated . This saturation is not accounted for in the method described in the EU RAR, which may very well account for the fact that the calculated concentration level is 10 times higher. In order to illustrate this, we have simulated the emission rate using our model. The emission rate for DEHP is plotted on a log scale. It starts at about 1 x $10^{-3.5}$ ug/m^2/s but drops to less than 1 x $10^{-4.5}$ug/m^2/s after approximately 140 days, and even further after that.

Furthermore, the "OLD" materials from 2001 contain more phthalates than the "NEW" materials from 2010 (ref. Danish EPA, 2001, resp. Danish EPA 2010) and are also emitting more phthalates to the air, according to the simulations made. This is in agreement with the findings based on earlier measurements that show higher concentrations of BBP and DEHP in dust from homes with floors covered with vinyl flooring.

Taken further to the risk characterisation we chose the "realistic worst case" gas phase level, resp. "realistic" gas phase level of phthalate (DEHP) concentration in air to be 0.81 µg/m^3 resp. 0.16 µg/m^3 taken from the table above. This is due to the fact that the by far dominating air concentration of phthalates originates from DEHP alone.

These values represent the concentration of DEHP in the gas phase. In order to compare the measured levels found in the literature with the air levels from the simulations it is necessary to

estimate the concentration of DEHP in particles in air, as the measured levels is the sum of gas phase and particles in air.

Weschler et al. (2008) estimated that about 80% of airborne DEHP in, for example, indoor settings are associated with particles (total suspended particles) in the air. Airborne particles increase the rate at which DEHP is transported between rooms by a factor 5 relative to gas-phase transport. DEHP also desorbs very rapidly from the particles. Both DBP and DEHP are to find among the SVOCs that typically have the highest airborne concentrations (sum of gas and particle phases). DEHP, DBP and BBP do belong to the group of SVOCs typically having the highest dust concentrations due to their relatively large abundance and low vapour pressure.

Based on a predicted indoor air DEHP concentration at steady state of 0.15 $\mu g/m^3$, Xu et al. (2009) predicted the particle DEHP concentration in the air at steady state to be 0.75 $\mu g/m^3$ (i.e. a 5-fold increase).

A "realistic worst case" scenario resp. "realistic" scenario concentration of DEHP in indoor air, at 20° C can be calculated using the steady state gas phase concentrations of 0.81 resp 0.16 $\mu g/m^3$. We assume 0.81 $\mu g/m^3$ to be a reasonable "worst case" gas phase level and 0.16 $\mu g/m^3$ to be a reasonable "realistic" level in a children´s play room in a relatively new building. Since the Weschler et al. (2008) study found that airborne particles increase the rate at which DEHP is transported between rooms by a factor 5 relative to gas-phase transport (i.e. 5 times more DEHP is bound to airborne particles than is to be found in the gas-phase of the indoor air), it can be inferred that (5 x 0.81=) 4 resp. (5 x 0.16=) 0.8 $\mu g/m^3$ are adsorbed to airborne particles. The total concentration of phthalates in air (the sum of gas phase concentration of DEHP and DEHP associated to airborne particles) may then be approximately (4 + 0.81 =) 4.8 resp. (0.8 + 0.16 =) 1 $\mu g/m^3$. These levels are 5-fold higher than the mean and maximum average levels found in the literature. Part of the difference may be due to the difference in furniture and coverings (PVC flooring and wallpaper).

Inhalation exposure through gas/vapour or particles in air can be estimated by the following equation:

$$\text{Gas/vapour/particles } (\mu g/kg \text{ bw/d}) = \frac{Y \times IR \times ED \times CFi}{Bw}$$

Y ($\mu g/m^3$) = phthalate concentration in inhaled air (air or particles in air)
IR (m^3/d) = inhalation rate
ED (hr/d) = exposure duration
CFi ((d/hr) = unit conversion factor of 1/24
BW (kg) = bodyweight

The calculation is based on the following assumptions:

	2-year olds	6/7-year olds	adults
Body weight (kg)	15.2	23.1	60
Respiration rate (m3/d)	7	14	18
Exposure duration (h/d)	21.93	20.73	19.32
Air conc., worst case (ug/m3)	4.8	4.8	4.8
Air conc., realistic (ug/m3)	1	1	1

Respirable fraction of inhaled substance, %	1	1	0.75

The respiration volumes are taken from REACH Guideline R15, Annex R15-5, table R15-1. The exposure durations are taken from US EPA- EFH, 2009, Table 16-21 and 16-22.

The simulated levels of DEHP above have been inserted in Table 30 and will be brought further to the risk characterisation.

B.9.3.2.3.4 Uncertainties on the measurements and estimation of exposure from indoor environment

The measurements of the exposure of phthalates from indoor air are based on simulations, calculations and measured data from the literature. The simulations are based on data from analysed articles. Due to limitations in the amount of available data, these are supplemented with default values and data from the literature. The exposure scenarios mainly involve phthalate containing articles with big surfaces. Due to the uncertainty connected with the limited amount of data, the exposure levels should be regarded as relatively conservative. The used data and defaults are further described earlier in this chapter and in more detail in Annex 1.

The measurements of the exposure of phthalates from dust are based on data from literature. The concentrations of phthalates in dust are expected to depend on the articles to be found in the indoor environment containing phthalates. If the studies of the content of phthalates in dust have been made in an indoor environment with a high number of articles containing phthalates this could overestimate the exposure, or the exposure could be underestimated if only a small number of articles containing phthalates can be found in the room.

B.9.3.2.4 Exposure from food

The exposure of the four ftalates from food is calculated based on literature data. As mentioned earlier only rather old data can be found in this area. It can therefore be expected that the levels of phthalates in food would be lower today, because one of the major source of phthalates in the food was considered to be from food contact materials, which are regulated today. A study by Gärtner et al. (2009) has analysed the migration of phthalates in infant food packed in recycled paperboard, and this study shows that phthalates and especially DIBP can still be found in infant food collected in the beginning of 2009. The concentrations are though considered as low and the median concentration in the food of 20.3 ng/g. The Danish authorities have made market surveillance on phthalates in food contact materials and these market surveillance results show that some food contact materials exceeds the limit for phthalates.

It can therefore still be expected that food will contain phthalates, due to the fact that one of the sources to phthalates in food is the environment, that some food contact materials will be able to migrate phthalates and market surveillance has shown that the limits are still exceeded from time to time. Due to the lack of more specific data on the contribution from the environmental compartments, the mentioned literature data are used to calculate the exposure from food. There are

no data that shows that the exposure from food is significantly lower today compared to the exposure calculated based on the older data.

In 2005, EFSA published opinions on DEHP, DBP and BBP for use in food contact materials. In their risk assessments they considered data on phthalate intake from food from two Danish studies by Petersen et al. (2000) and Müller et al. (2003) and a British study by MAFF (1996). In Table 27 these studies are compared to other studies on phthalate intake from food published after the EFSA opinions.

Internal exposure estimates are necessary for the cumulative risk assessment of phthalates in the current report. Most of the studies listed in Table 27 are based on dietary measurements, and no conversion factors from external to internal doses are described in the relevant papers. To convert these external exposure estimates to internal exposure estimates, the oral absorption factors listed in Table 15 have been applied. For DBP, DIBP and BBP, and for DEHP in children, an absorption factor of 100 % has been applied (i.e. internal dose = external dose), while for DEHP an absorption factor of 50 % has been applied for adults. The study by Wormuth et al. (2006), is based on concentration measurement of phthalates in food, and exposure measurements include a conversion to internal exposure estimates by applying oral uptake rates (around 70 %).

The gray-shaded data in Table 27 are transferred to Table 31 collecting "realistic worst case" data on exposure from food and dust. Data from Müller et al. (2003), are selected as realistic median/mean estimates, as these estimates are relatively high but close to other median estimates, and as these data are used in the EFSA opinions. The estimates listed in Table 27 are based on the mean daily intake estimates from Petersen & Breindahl (2000), calculated from measurements of phthalates in total diet samples (mean of 29 total diet samples).

It should be noted, that Petersen & Breindahl presented mean values instead of medians as presented for other studies. In addition to mean values, Petersen & Breindahl (2000), present intake estimates based on the highest measured phthalate concentrations in a total diet sample. These highest intake estimates are selected as 95^{th} percentile values ("realistic worst case") for infants and adults (Petersen & Breindahl, 2000). The study by Wormuth et al. (2006) is the only study reporting 95^{th} percentile values for children around 6/7-year old (age 4-10), and these values are relatively close to median values in the Müller et al. (2003) study and are selected as "realistic worst case" 95^{th} percentile values for children. As the study by Wormuth et al. (2006), is the only study giving specific values on DIBP in the relevant age group, data from this study are used for further comparison (gray-shaded in table 25 and transferred to Table 31). Details on the applied studies are described in the following.

In 2003, the Danish Veterinary and Food Administration published a report on "Human exposure to selected phthalates in Denmark" by Müller et al. (2003) who compared exposure to five phthalates (DEHP, DBP, BBP, DINP, DIDP) from foods, environment and consumer products (Müller et al., 2003). The study showed that food is the dominant pathway of exposure for all age groups, and that toys also contributed significantly to DEHP exposure in young children. The computer program EUSES was used to make a simple and a refined estimate of phthalate exposure, with the refined method including measured levels of phthalates in environment and food samples.

The predicted intake of phthalates in food based on the simple method was lower than measured concentrations of DEHP and DBP in foods, and as measurements of DEHP were available, these

were used in a refined estimate of DEHP exposure. A specific estimate for food was made only for DEHP (Table 27). For the other phthalates table 25 includes refined "total daily intake" estimates, which are thus interpreted as total daily intake via food. The refined estimate for DEHP including measured concentrations in foods and environment is reported to be similar to intake estimates based on foods and to the daily intake estimate reported in the EU RAR for DEHP. Also for DBP and BBP the refined estimates (based on environmental concentrations) are considered to be similar to exposure estimates from other studies, (Müller et al., 2003).

Petersen & Breindahl (2000), and Fromme et al. (2007), calculated daily intake levels based on phthalate measurements in duplicate diet samples. These intake levels based on dietary levels have been divided by body weight of the relevant age group. To describe intake of 2-year olds, intake values of adults (per kg bw) have been multiplied by two, as 2-year olds have twice the energy intake of adults per kg bodyweight (Danish EPA, 2009). Petersen & Breindahl (2000), calculated mean daily intakes of adults as well as the highest daily intake value (highest daily intake of 29 samples). In table 25 the mean value is listed as "median" and the highest intake value is listed as "95th percentile". In some cases, the use of mean values instead of median values may lead to an overestimation of typical exposures.

Wormuth et al. (2006) based their estimates of phthalate exposure from foods on concentration measurements published in 1995 to 2002. The authors concluded that the calculated exposure estimates were comparable to exposure estimates based on urinary levels. Food was considered the main source of DIBP, DBP and DEHP accounting for 55-60 % of total exposure to toddlers (1-3 years, 13 kg bw) and 90-100 % for adults. For BBP, only 18 % of intake was from food in toddlers and around 65 % was from food in adults. In the Wormuth paper, exposure from food is reported as a percentage of total exposure together with total exposure estimates in µg/kg bw/day. Thus, the listed estimates on exposure from food alone are calculated by multiplying total exposure with the food-fraction. An example for DIBP in children: median "daily internal exposure" is 0.3 µg/kg bw/day of which 65 % is from food which is estimated to => 0.2 µg/kg bw/day from food.

Analysis of DEHP from 164 meals and snacks from 10 German boys in the age 5 to 8 years were collected in 2005. These meals were exact duplicates of the boys' intake of food. The concentration of DEHP in the meals were between 10.00 and 1510.00 µg/kg with a median concentration of 35.20 µg/kg (personal communication UBA, 2011).

The EFSA opinions on phthalates for use in food contact materials do not single out one specific estimate for phthalate exposure via food but refer to a number of studies (The EFSA Journal, DBP, 2005; The EFSA Journal, DEHP, 2005; The EFSA Journal, BBP, 2005).

The EU Risk assessment reports reported specific estimates for exposure via food:

- The EU RAR for DEHP used an exposure estimate of 19 µg/kg bw/day for children and 2 µg/kg bw/day for adults based on an EUSES estimate (these estimates are for food, water and air with main contribution from food, and these estimates are different from the EUSES estimates reported in Müller et al. (2003)). These levels are comparable to values selected in Table 27.

- The EU RAR for DBP used an exposure estimate of 27 µg/kg bw/day for adults based on MAFF (1987). This estimate is higher than the values in Table 27 including the more recent data from MAFF (1996).

- The EU RAR for BBP used an exposure estimate of 0.8 µg/kg bw/day for children and 0.3 µg/kg bw/day for adults based on the data from MAFF (1996) listed in Table 27. These estimates are lower than the exposure estimates selected here from Müller et al. (2003) and Petersen & Breindahl (2000). The estimates from Müller et al. (2003) are selected as these are the values used by EFSA for the median daily intake, and the estimates from Petersen & Breindahl (2000) were selected as these reports the 95^{th} percentile.

Table 27 Intake estimates for phthalates from food. Gray-shaded values are selected for comparison of "realistic worst case" estimates in Table 31.

Intake from food in µg/kg bw/day				**Toddler[#]**		**Child[##]**		**Adult**	
Study	**Region**	**Median (µg/day)**	**95-p (µg/day)**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**
DEHP (external)									
Fromme et al., 2007,	Germany	162	309	4.8	8			2.4	4.0
MAFF 1996 in The EFSA Journal, DEHP	UK	150		5	10			2.5	5
Müller et al., 2003,	Europe			26		11		4.5	
Petersen & Breindahl, 2000,	Denmark	300	1100	8.6	31			4.3	15.7
Tsumura et al., 2003	Japan	160		5				2.5	
Personal communication UBA, 2011	Germany					1.27	3.17		
DEHP (internal)									
Fromme et al., 2007	Germany	162	309	4.8	8			1.2	2.0
MAFF, 1996 in The EFSA Journal, DEHP	UK	150		5	10			1.3	2.5
Müller et al., 2003,	Europe			26		11		2.3	
Petersen & Breindahl, 2000,	Denmark	300	1100	8.6	31			2.2	7.8
Tsumura et al., 2003,	Japan	160		5				1.3	
Personal communication UBA, 2011	Germany					1.27	3.17		
Wormuth et al., 2006	EU, US, Asia			4.4	44	1.6	16	2.7	
DBP (internal=external)									

Intake from food in µg/kg bw/day				Toddler[#]		Child[##]		Adult	
Study	**Region**	**Median (µg/day)**	**95-p (µg/day)**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**	**Median daily intake**	**95-p daily intake**
Fromme et al., 2007	Germany	16	91	0.5	3.2			0.3	1.4
MAFF 1996 in the EFSA Journal, DBP (2005)	UK	13	31	0.4	1.0			0.2	0.5
Müller et al., 2003,	Europe			8.0		3.5		1.6	
Petersen & Breindahl, 2000	Denmark	290	720	8.2	20			4.1	10.2
Tsumura et al., 2003	Japan	13		0.4				0.2	
Wormuth et al., 2006	EU, US, Asia			2.2	22	0.7	10	3.1	
DIBP (internal=external)									
Fromme et al., 2007	Germany	42	157	1.1	4.2			0.6	2.1
Wormuth et al., 2006	EU, US, Asia			0.48	2.4	0.2	1.0	0.5	1.5
BBP (internal=external)									
Fromme et al., 2007	Germany	15	25	0.5	0.8			0.2	0.4
MAFF, 1996 in the EFSA Journal, BBP (2005)	UK	8	20	0.3	0.6			0.1	0.3
Müller et al., 2003	Europe			5.9		2.4		1.0	
Petersen & Breindahl, 2000,	Denmark	30	320	1.0	9			0.5	4.5
Tsumura et al., 2003	Japan	3.4		0.12				0.06	
Wormuth et al., 2006	EU, US, Asia			0.07	1.1	0.03	0.8	0.2	

[#] Study Wormuth et al. (2006) and Müller et al. (2003) include specific data for toddlers (age 1-3 and 1-6, respectively), but in all other studies phthalate intake of toddlers is calculated as twice the estimate for adults, as 2-year olds have twice the energy intake of adults per kg bodyweight (Danish EPA, 2009).

[##]Phthalate intake of children around 6/7-years of age is only listed for study (Wormuth et al., 2006), which includes specific data for 4-10 year olds, and for study (Müller et al., 2003), which provides estimates for 7-14 year-olds, which used as approximate estimates for 6/7-year olds.

B.9.3.2.4.1 Uncertainties on the measurements of exposure from food

The exposure of phthalates from food is expected to be overestimated as mentioned above because of the lack of new data. It is expected that the exposure of phthalates in food is reduced due to the regulation of phthalates in food contact materials.

It is, however, expected that there still will be an exposure from food as one of the sources of phthalates in food is phthalates from the environment. Phthalates in food from the environment will originate from the production of articles containing phthalates, from the use of articles and when the articles end up in the waste stream, but there is no information of the size of the source. There are limited data on the migration of phthalate from plasticized PVC into environmental media. However, due the total volume of plasticized PVC produced, it is possible that PVC or other polymer/polymericlike materials containing phthalates may be long-term and dispersive sources of human and environmental exposures to phthalates (US EPA 2009).

Even though the exposure of phthalates via food is expected to be overestimated by using the levels from literature; these are however the only data that can be found and are therefore used. There are no data that shows that the exposure from food is significantly lower today compared to the exposure calculated based on the older data. But a study by Gärtner et al. (2009) has analysed the migration of phthalates in infant food packed in recycled paperboard, and this study shows that phthalates and especially DIBP can still be found in infant food collected in the beginning of 2009. The concentrations are though considered as low and the median concentration in the food of 20.3 ng/g. The Danish authorities have made market surveillance on phthalates in food contact materials and these market surveillance results show that some food contact materials exceeds the limit for phthalates. It can therefore still be expected that food will contain phthalates, due to the fact that one of the sources to phthalates in food is the environment, that some food contact materials will be able to migrate phthalates and market surveillance has shown that the limits are still exceeded from time to time.

B 9.3.2.5 Indirect exposure of humans via the environment

Not relevant

B.9.3.2.6 Other sources (for example natural sources, unintentional releases)

Not relevant

B.9.3.2.7 Exposure based on biomonitoring data

In order to see if the exposure levels estimated by calculation and simulation is in agreement with biomonitoring data an information retrieval has been performed. Biomonitoring data are examples of the levels of phthalates that the population has been exposed to, and biomonitoring data can therefore contribute to the validation of the calculated exposure from food, indoor environment and articles.

Table 28 lists studies on exposure estimates based on biomonitoring, i.e. measurements of phthalate metabolites in urine from human study populations of different ages. The calculated exposure estimates are thus to be interpreted as internal doses. For each age group, a value representing a "realistic worst case" estimate is selected together with a realistic "high median" value (gray-shaded) and transferred to Table 32 and Table 33 comparing exposure estimates from biomonitoring to exposure estimates from foods and dust.

Most studies are based on adults, a few studies are on children older than 6 years and only one study presents data on 2 to 4 year old infants. Table 28 only includes studies reporting exposure estimates based on measured urinary concentration levels. Exposure estimates depend on the method of calculation, as exposure estimates based on urinary volume are generally higher than exposure estimates based on urinary creatinine levels. The method based on urinary creatinine adjusted concentrations takes account of the daily urinary excretion depending on gender and height of the individual. The method based on volume related concentration takes account of the urine volume depending on age and bodyweight of the individual. The values obtained by the volume based calculation method were found to be between 1.5 to 2 times higher than values obtained with the creatinine based method (Koch et al., 2007). Koch et al. (2007) indicate that creatinine adjustment – which is used in most of the published studies – may underestimate the daily phthalate intake. In line with this, Wittassek et al. (2007) report that using creatinine or volume based estimates is equally probable. Therefore, volume based estimates are used for selection of "realistic worst case" exposure in the current report. Measurements from the same studies are used by several authors to make slightly different exposure estimates due to different methods of calculation.

DEHP: Wittassek et al. (2007a) present exposure estimates for 2-4 year olds and 5-6 year olds , and these values (gray-shaded in Table 28) are selected as representative for "realistic worst case" exposure to DEHP for toddlers and infants to be compared to other exposure data later in this report. For adults, data from Wittasek et al. (2007b) are used (greyshaded in Table 28). Early studies on DEHP levels in urine are based on measurements of the metabolite mono-2-ethylhexyl phthalate (MEHP) only. After 2004, studies include measurements of secondary metabolites to MEHP, namely mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) (also called 5-OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) (also called 5-oxo-MEHP) and Mon*o*-(2-ethyl-5-carboxypentyl) phthalate (MECPP) (also called 5cx-MEHP). When these secondary metabolites are measured, exposure estimates are higher than in studies using only MEHP measurements, and there is scientific agreement that secondary DEHP metabolites are superior biomonitoring markers and therefore preferred to estimates based on MEHP only, as described by Koch et al. (2005).

DBP, DIBP and BBP: Only the study by Koch et al. (2007) includes specific data on children, and the listed exposure values for DBP and BBP (gray-shaded in Table 28) are selected for the comparison to other types of exposure estimates. For adults, there is good agreement between the studies on DBP and exposure values for DBP and DIBP from Wittassek et al. (2007b) are selected for further comparisons. For BBP, the study by Wittassek et al. (2007b) showed lower exposures than the other studies listed in the same paper. The data by Kohn et al. (2000), reviewed by Wittassek et al. (2007b), are selected for further comparison (gray-shaded in Table 28).

Some of the exposure estimates listed in Table 28 is adopted from two review papers (Koch & Calafat, 2009; Calafat & McKee, 2006). Koch & Calafat (2009) reviewed biomonitoring data from Germany and United States and concluded that phthalate exposures are within the same order of

magnitude in these countries, but that BBP was highest in US, while DIBP was highest in Germany (Koch & Calafat, 2009).

In Germany, declining levels of DEHP, DBP and BBP and increasing levels of DINP seemed to agree with the restrictions of use of certain phthalates within recent years (Koch & Calafat, 2009). Calafat & McKee (2006) compared different biomonitoring-based exposure estimates and indirect estimates and found geometric mean DEHP exposures around 2-3 µg/kg bw/day (their Table 4) with 95th percentile values from 12 to 17 µg/kg bw/day for children and adults (Calafat & McKee, 2006). They found that biomonitoring data gave slightly lower estimates than indirect estimates based on phthalate levels in food, dust etc. Their comparison of adults and children concluded similar exposures for adults and children based on biomonitoring, but revealed higher indirect estimates in children than adults, a finding that is supported by other studies.

A biomonitoring study made by Wittassek et al. (2009), have measured the content of metabolites of DEHP, DBP, DIBP and BBP in 11 pairs of amniotic fluid and corresponding maternal urine. This study shows that oxidative phthalate metabolites are found in both amniotic fluid and the corresponding maternal urine, with generally much higher concentration in the maternal urine. The urine samples are probably contaminated with DEHP from the PVC urine bags that contained 20-40 % DEHP, giving level of DEHP metabolites in the maternal urine that must be considered too high.

The grayshaded values in Table 28 are used futher in the calculation of the risk characterization based on biomonitoring data. The measured exposure from biomonitoring data can be seen as a real picture of the level of phthalates that the population has been exposed to. The biomonitoring data generally shows that the exposure concentrations are higher in children than in adults.This is probably due to children´s higher intake of food and dust in combination with their lower body weight and their different behaviour, for example a larger tendency to mouth articles.

B.9.3.2.7.1 Uncertainties on exposure based on biomonitoring data

The biomonitoring data are measured before the legislation of phthalates in food contact materials entered into force in 2008, but after the voluntary agreement on the phasing out of phthalates in tubes for milk and foils for food. The data are from before the EU ban of some phthalates in toys and childcare articles from 2007 (REACH Regulation annex XVII). Before the EU ban of phthalates in 2007, an emergency ban on 6 phthalates in toys and childcare articles for children less than 3 years of age were in force. Even though legislation has entered into force after the measurements of the biomonitoring data, attention were already on phthalates at the time of the measurements. There could therefore be an overestimation of the exposure based on biomonitoring data, but as there has been a high attention on the substitution of these four phthalates for several years, it is not expected that a possible overestimation would be high.

Table 28 Exposure estimates based on biomonitoring in µg/kg bw/day. Volume: indicates exposure estimates based on urinary volume; creatinine: indicates exposure estimates based on urinary creatinine levels. Gray-shaded values are selected for comparison of "realistic worst case" estimates in Table 32.

Reference	Metabolite	Age, year	Median[a]	95th percentile	Range	Notes
DEHP						
Becker et al., 2004 reported in Calafat & McKee, 2006	MEHP/MEHHP/MEOHP	Children 3-14, 2001/2002	0.7/2.6/3.1	2.8/10.7/11.7		
Blount et al., 2000 reported in Calafat & McKee, 2006	MEHP-based only	289 adults, 1988-94	0.5	3.3		
CDC 2005 reported in Calafat & McKee, 2006	MEHP/MEHHP/MEOHP	Age 6 to adult Children 6-11	0.9/2.1/2.2 0.6/2.4/2.6	7.1/16.8/15.6 3.7/13.2/12.8		
David, 2000 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Matsumoto et al., 2008	MEHP-based only	Adult	0.6	3.1		
Koch et al., 2003 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Koch & Calafat, 2009		Child + Adult, 2002	4.6	17.0	58.2-166	
Koch et al., 2003 recalculated in Calafat & McKee, 2006	MEHP/MEHHP/MEOHP	85 age 7 to 63, 2002	2.7 /6.5/7.4	7.5/16.3/18.9		
Koch et al., 2003 recalculated in Matsumoto et al., 2008	Recalculated MEHP/MEHHP/MEOHP	85 age 7 to 63, 2002	10.3/13.5/14.2	38.3/51.4/52.8		Higher than other calculations on same data
Koch et al., 2004 reported in Calafat & McKee, 2006	MEHP/MEHHP/MEOHP	36 children <7, 2003	1.0/3.5/3.8	3.3/7.1/7.4		
Koch et al., 2011	MEHP/MEHHP/MEOHP/ MECPP/2cx-MMHP	108 children age 5-6	4,5	18.0		2007 values
Kohn et al., 2000 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Koch & Calafat, 2009		Adult	0.7	3.6		Same data as [52] and [53] but different calculation
Silva et al., 2004 reported in Calafat & McKee, 2006	MEHP-based only	2536 persons age 6 to >20 Children 6-11, 1999-2000	0.7 0.6	4.0 5.0		
Wittassek et al., 2007a	Intake calculations on data from Becker et al. (2004)	2-4 years 5-6 years, 2001-	10.7 (5.7) 10.0 (6.1)	45.0 (23.4) 19.4 (14.7)	0.4-409 (1.8-140) 2.9-43.7 (1.3-28.8)	Volume (Creatinine) Volume (Creatinine)

Reference	Metabolite	Age, year	Median[a]	95th percentile	Range	Notes
		2002				
Wittassek et al., 2007b	5 metabolites	Adult, 2001/2003	2.7	6.4	0.82-7.1	2001/2003 values
Personal communication UBA, 2011	MEHP/MEHHP/MEOHP/ MECPP/2cx-MMHP,	10 boys age 5 to 8	2.27	7.02	0.78-12.73	2005 values
DBP/DIBP						
	Metabolite	Age	Median	95th percentile	Range	
David, 2000 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Matsumoto et al., 2008		Adult	1.6	6.9		DIBP+DBP
Koch et al., 2003 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Koch & Calafat, 2009		Child + Adult, 2002	5.2	16.2	Max 58.2 to 166	Different conversion factors
Koch et al., 2007		Children 4- 11, 2001-2002	7.6 (4.1)	30.5 (14.9)	0.91-110 (0.66-76.4)	Volume (Creatinine)
Koch et al., 2011	MnBP	108 children age 5-6	1.9	6.4		2007 values
Koch et al., 2011	MIBP	108 children age 5-6	2.1	11.0		2007 values
Kohn et al., 2000 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Koch & Calafat, 2009		Adult	1.5	7.2		DIBP+DBP
Wittassek et al., 2007b	MnBP MiBP	Adult, age 20-29 Adult, age 20-29, 2001/2003	2.2 1.5	7.3 4.2	0.49-116 0.29-12.6	DBP, 2001/2003 DIBP, 2001/2003
BBP						
David, 2000 reported in Wittassek et al., 2007a ; Wittassek et al., 2007b; Matsumoto et al., 2008		Adult	0.73	3.3		
Koch et al., 2003 reported in Wittassek et al., 2007a ;		Child + Adult, 2002	0.6	2.5		

Reference	Metabolite	Age, year	Median[a]	95th percentile	Range	Notes
Wittassek et al., 2007b.						
Koch et al., 2007		Children 4-11, 2001-2002	0.77 (0.42)	4.5 (2.6)	0.05-31.3 (0.06-13.9)	Volume (Creatinine)
Koch et al., 2011	MBP	108 children age 5-6	0.3	2.6		2007 values
Kohn et al., 2000 reported in Wittassek et al., 2007a; Wittassek et al., 2007b		Adult	0.88	4.0		
Wittassek et al., 2007b		Adult, 2001/2003	0.22	0.75	0.02-1.74	2001/2003 values

B.9.3.2.8 Combined human exposure assessment

A not yet published study from Germany (referenced as "Personal communication UBA, 2011" in Table 23, Table 27 and Table 28) has measured the content of 5 DEHP metabolites in urinary samples from 10 boys in the age 5 to 8 years. At the same time the content of DEHP in the air and dust from their homes were measured and a duplicate sample of their food and drinks were taken and anlysed for DEHP as well. A comparison of the calculated exposure from food and indoor environment to the biomonitoring data shows that the exposure from indoor environment is probably overestimated. The study concludes that 58 % of the daily DEHP intake originates from foodstuff, dust contributes to 18 % of the daily DEHP intake (model calculations) and that it cannot be excluded that the TDI (48 mg/kg bw/day) can be exeeded occasionally. The conclusions from this study support other data gathered in this dossier. It should though be noted that the samples were taken before the new legislation on food contact materials and the exposure from food therefore might be lower today.

See B. 10 - Risk characterisation

B.10 Risk characterization

B.10.1.Human health

B.10.1.1 Risk characterization based on background exposure from articles, indoor environment and food

In the current dossier, the dose addition principle is applied to summarize the risk of cumulative phthalate exposure by adding risk characterization ratios (RCRs). The RCR for a chemical is defined as the ratio between exposure level and DNEL (ECHA part E, 2008). The RCR is calculated as the ratio between the internal exposure estimates and the internal DNEL for the individual phthalates, as described in section B.5.11.

$$RCR = \frac{Exposure}{DNEL}$$

If the RCR for a single chemical exceeds 1, i.e. when exposure exceeds DNEL, it may be concluded that the risk is not controlled (ECHA part E, 2008). In a situation with exposure to several similarly acting chemicals, the dose addition principle will imply that a cumulative RCR can be calculated by adding the RCR for each chemical, see B.1.4.2. When this cumulative RCR exceeds 1 the risk is considered not to be controlled for the chemicals included in the cumulative RCR.

The exposure and the risk are calculated for the three different population groups:

- 2-years old
- 6/7-years old
- Adults

The reasoning for this is the different use and behavior of the three groups. It can for example be expected that 2-year olds will have a tendency to mouth articles, while 6/7-years old are not

expected to mouth that many articles, but they will have dermal contact to other articles compaired to adults.

B.10.1.2 Occupational exposure

Workers can be exposed to the four phthalates during manufacturing of articles – not only due to direct "hands on" contact, but also due to the emissions from e.g. industrial extrusion processes or the presence of articles like e.g. PVC flooring at the production site. Other occupational exposures can come from different job situations in private households, nurseries, offices, hospitals, kinder gardens etc. Examples are the emissions to the indoor air from floorings, wallpaper, curtains, carpet tiles, tablecloths and curtains etc. In this regard the same calculations and reasoning given for consumers can be used for the estimation of occupational exposure though considering the special protection prescribed in the regulations limiting the occupational exposure with specific instructions, use of PPE´s, established local exhaust and ventilation, etc.

B.10.1.3 Consumers

See B.10.1.5

B.10.1.4 Indirect exposure of humans via the environment

See B.10.1.5

B.10.1.5 Humans exposed to articles, food and indoor environment

The exposure data on food, indoor environment, articles and biomonitoring, from Table 20, Table 22, Table 23, Table 27 and Table 28 are used to calculate the RCR values. The 95th percentile estimates are considered to be "realistic worst case exposure" levels. The "high median" exposure estimates were selected among the studies listed in Table 23, Table 27 and Table 28 as high median value of exposure to each phthalate from each source. In order to make a scenario representing lower exposures, we selected the lowest median value of the studies listed in Table 23, Table 27 and Table 28 to each phthalate from each source. These values are listed as "lowest median" exposure estimates to each phthalate from each source.

B.10.1.5.1 Articles

For articles the same exposure value is used for low, median and high median and realistic worst case. The only exceptions are sex toys for adults, erasers for 6/7-year olds and plastic sandals for all age groups. For these articles a realistic worst case scenario exposure is calculated as described in section *B.9.3.2.2 Exposure from articles.*

The exposure values for articles can be found in Table 20 and Table 22. The estimated exposure values and RCR-values for articles are derived from the sum of the individual phthalates from all the articles described in Table 20 and Table 22. This will probably be an overestimation of the exposure from articles especially for the worst case scenario as it might not be realistic that all articles will be used in the expected way every day. The estimated exposure and RCR values for each phthalate for articles are given in Table 29.

Table 29 Exposure and RCR for all articles.

Internal intake estimates, µg/kg bw/day	Age	Median (lowest)	Median (selected high value)	95th percentile ("realistic worst case")	DNEL, µg/kg bw/day	RCR based on lowest median	RCR based on high median	RCR based on 95th percentiles
Estimated exposure (oral + dermal) from articles*, from Table 20 and Table 22								

Internal intake estimates, µg/kg bw/day	Age	Median (lowest)	Median (selected high value)	95th percentile ("realistic worst case")	DNEL, µg/kg bw/day	RCR based on lowest median	RCR based on high median	RCR based on 95th percentiles
DEHP	2-year old 6/7-year old Adult	1.7096 18.4686 1.3478	1.7096 18.4686 1.3478	4.4289 176.80 19.89	25	0.068 0.7387 0.054	0.068 0.7387 0.054	0.1771 7.07 0.7956
DBP	2-year old 6/7-year old Adult	0 0 0.8629	0 0 0.8629	0 3.9076 5.4971	6.7	0 0 0.1288	0 0 0.1288	0 0.58 0.8205
DIBP	2-year old 6/7-year old Adult	1.5062 1.0151 5.5109	1.5062 1.0151 5.5109	5.0640 1.0151 3.2019	1250	0.0012 0.0008 0.0044	0.0012 0.0008 0.0044	0.0041 0.0008 0.0026
Articles total	2-year old 6/7-year old Adult	**3.2158** **19.4837** **7.7216**	**3.2158** **19.4837** **7.7216**	**9.4929** **181.72** **28.589**		**0.07** **0.74** **0.19**	**0.07** **0.74** **0.19**	**0.18** **7.65** **1.62**

* The calculated exposure from all the articles (Table 20 and Table 22).

The general picture in table 26 is that DEHP will be the main contributor to the total RCR for the four phthalates for articles. Especially in relation to adults, DBP will also take part in the contribution to the total RCR. This is due to the exposure from plastic sandals and the low DNEL for DBP.

RCR from mouthing erasers is 0.63 (mouthing an eraser 1 hour per day, median 6/7-year old) (based on data from Danish EPA, 2007) and the total RCR for articles is 0.74. The exposure from mouthing erasers 1 hour per day will therefore contribute to the total RCR to a very high extend. If it is assumed that the eraser is only mouthed 10 minutes per day the RCR contribution from erasers would be 0.1. For articles it can be seen that exposure to single articles with a high concentration of phthalates, as for example erasers, contributes to a high exposure to the substances. This can be seen from the exposure estimates from articles in Table 20 and Table 22.

The use of sex toys contributes with a RCR value of 0.73 in the worst case scenario for adults. Here it is assumed that an oil based lubricant is used. This results in a migration which is more than a factor 900 times higher than without the use of an oil based lubricant. The assumptions made for the exposure scenario therefore have a large impact on the RCR.

The total RCR values for articles shows that in the worst case scenario a risk is identified for 6/7-year olds and adults. It would normally be expected that the highest risk would be for the 2-year olds due to the lower bodyweight, but in this case the opposite picture is observed. This is due to single products like erasers, a pair of plastic sandals and sex toys for adults showing a high content and migration of phthalates. This can be seen from the exposure estimates from articles in Table 20 and Table 22.

B.10.1.5.2 Indoor environment

The estimated exposure values for the four phthalates in indoor air and dust is given in Table 30 below together with the RCR values for each phthalate. The exposure values for dust and indoor air can be found in Table 23 and section B.9.3.2.3.2 Exposure to phthalates from indoor air. Only median and worst case exposure simulations are made and the exposure and RCR for lowest and high median are therefore identical.

Table 30 Exposure and RCR from indoor environment.

Internal intake estimates, µg/kg bw/day	Age	Median (lowest)	Median (selected high value)	95th percentile ("realistic worst case")	DNEL, µg/kg bw/day	RCR based on lowest median	RCR based on high median	RCR based 95th percentiles
Estimated exposure from indoor dust, from Table 23[a]								
DEHP	2-year old	2.24	5.1	26.8	25	0.09	0.20	1.1
	6/7-year old	1.47	3.3	17.6		0.06	0.13	0.7
	Adult	0.14	0.3	1.7		0.006	0.01	0.1
DBP	2-year old	0.13	1.0	3.7	6.7	0.02	0.15	0.55
	6/7-year old	0.09	0.7	2.5		0.01	0.10	0.37
	Adult	0.02	0.13	0.5		0.003	0.02	0.07
DIBP	2-year old	0.013	0.3	2.0	1250	0.00001	0.0002	0.0016
	6/7-year old	0.008	0.2	1.3		0.00001	0.0002	0.0010
	Adult	0.002	0.04	0.3		0.000002	0.00003	0.0002
BBP	2-year old	0.10	0.9	3.9	500	0.0002	0.002	0.0078
	6/7-year old	0.06	0.6	2.6		0.0001	0.001	0.0052
	Adult	0.01	0.1	0.5		0.00002	0.0002	0.0010
Dust total	2-year old	**2.5**	**7.3**	**36.5**		**0.11**	**0.36**	**1.63**
	6/7-year old	**1.6**	**4.8**	**24**		**0.072**	**0.24**	**1.08**
	Adult	**0.2**	**0.6**	**2.9**		**0.009**	**0.032**	**0.14**
Simulated exposures from particles in air and air in indoor environment, from section B.9.3.2.3.2 Exposure to phthalates fr indoor air								
DEHP, air	2-year old	0.04	0.04	0.2	25	0.002	0.002	0.01
	6/7-year old	0.05	0.05	0.3		0.002	0.002	0.01
	Adult	0.008	0.008	0.04		0.0003	0.0003	0.002
DEHP particles in air	2-year old	0.2	0.2	1.0	25	0.008	0.008	0.04
	6/7-year old	0.3	0.3	1.3		0.01	0.01	0.05
	Adult	0.04	0.04	0.2		0.002	0.002	0.008
DEHP indoor air total	2-year old	**0.24**	**0.24**	**1.2**	**25**	**0.01**	**0.01**	**0.05**
	6/7-year old	**0.35**	**0.35**	**1.6**		**0.012**	**0.012**	**0.06**
	Adult	**0.05**	**0.05**	**0.24**		**0.0023**	**0.0023**	**0.01**
Indoor environment total	**2-year old**	**2.74**	**7.54**	**37.7**		**0.12**	**0.37**	**1.68**
	6/7-year old	**1.95**	**5.15**	**25.6**		**0.084**	**0.252**	**1.14**
	Adult	**0.25**	**0.65**	**3.14**		**0.0113**	**0.0343**	**0.15**

[a] Estimated exposure levels from dust are based on dust measurements (Bornehag et al., 2005) multiplied by dust intake and divided by body weight

In general the calculated RCRs for indoor air in Table 30 indicate that the risk of exposure to phthalates originating from the indoor air is limited. However, even though the direct exposure from airborne phthalates may be limited as expressed by the RCR value, the content in air have consequences for building up phthalates levels in the indoor environment on surfaces and dust, which then further contribute to the indirect exposure. Thus there are arguments for the control and limiting of the sources that generate air emissions of phthalates to indoor air.

From Table 30 it can be seen that DEHP and DBP is responsible for the major contribution to the total RCR for indoor environment. Not surprisingly, dust contains the largest amounts of phthalates compared to the concentrations in the air (gas phase and particles in air), and the total RCR from dust alone based on 95th percentiles shows a risk for 2-year olds and 6/7-year olds. The RCR based on low and high median values are below 1 for all age groups, but there will be an exposure from the indoor environment as long as articles containing phthalates can be found in the indoor environment.

There are clear indications that a reduction in DEHP concentration in articles, in the indoor environment, results in lower concentration of DEHP in the indoor air. The simulations on the concentrations in indoor air show that the use of wallpaper and floorings with a lower content of

phthalates as expected results in lower concentrations of phthalates in the indoor air. This is in agreement with a previous finding in a review by Koch & Calafat (2009).

B.10.1.5.3 Food

The estimated exposure values for the four phthalates from food are given in Table 31 below together with the RCR values for each phthalate. The exposure values for food can be found in Table 27.

Table 31 Exposure and risk from food (from table 25).

Internal intake estimates µg/kg bw/day	Age	Median (lowest)	Median (selected high value)	95th percentile ("realistic worst case")	DNEL, µg/kg bw/day	RCR based on lowest median	RCR based on high median	RCR based on 95th percentiles
DEHP	2-year old [a]	4.4	26	31	25	0.18	1.0	1.24
	6/7-year old [b]	1.6	11	16		0.06	0.4	0.64
	Adult	1.2	2.2	8		0.05	0.1	0.32
DBP	2-year old [a]	0.4	8.0	20	6.7	0.06	1.2	3.0
	6/7-year old [b]	0.7	3.5	10		0.10	0.5	1.5
	Adult	0.2	1.6	10		0.03	0.2	1.5
DIBP	2-year old	0.05	0.5	2.4	1250	0.00004	0.0004	0.002
	6/7-year old	0.2	0.2	1.0		0.0002	0.0002	0.001
	Adult	0.5	0.5	1.5		0.0004	0.0004	0.001
BBP	2-year old [a]	0.07	5.9	9	500	0.0001	0.012	0.018
	6/7-year old [b]	0.03	2.4	0.8		0.0001	0.005	0.005[c]
	Adult	0.06	0.5	4.5		0.0001	0.001	0.009
Food total	**2-year old**	**4.9**	**40**	**62**		**0.24**	**2.2**	**4.24**
	6/7-year old	**2.5**	**17**	**28**		**0.17**	**0.97**	**2.13**
	Adult	**2.0**	**4.8**	**24**		**0.078**	**0.33**	**1.82**

[a] Estimated exposure levels from food in toddlers are based on median values for 1-6 year olds (Müller et al., 2003) and 95th percentile estimates calculated as twice the adult estimates of high phthalate intake (Petersen & Breindahl, 2000).

[b] Estimated exposure levels from food in children are based on median values for 7-14 year olds (Müller et al., 2003) and 95th percentile estimates for 4-10 year olds (Wormuth et al., 2006).

[c] Based on the highest of the two listed values for children's exposure to BBP from food, i.e. median estimate from Müller et al.,(2003).

The RCR values for food show that the exposures to phthalates are primarily due to exposure to DEHP and DBP. The total RCR for food indicate that food alone can pose a risk for all age groups for the 95th percentile and for 2-year olds based on high median exposures. The RCR for the 6/7 years old are very close to 1 (0.97). It should be emphasized that the exposure data on the four phthalates from food may be overestimated, as these are old data from before the legislation of phthalates in the food contact material entered into force. It is assumed that there still will be an exposure to phthalates via food, as the food is contaminated with phthalates from the environment.

B.10.1.5.4 Biomonitoring

The exposure values for the four phthalates based on biomonitoring data are given in Table 32 below together with the RCR values for each phthalate. The exposure values for biomonitoring can be found in Table 28 and are based on measurements of phthalate metabolites in urine from human population as described in section B.9.3.2.7 Exposure based on biomonitoring data.

Table 32 Exposure and RCR based on biomonitoring data (from table 26).

Internal exposure estimates, µg/kg bw/day	Age	Median (lowest)	Median (selected high value)	95th percentile ("realistic worst case")	DNEL, µg/kg bw/day	RCR based on lowest median	RCR based on high median	RCR based on 95th percentiles
DEHP	Toddler 2-4y Child 5-6y Adult	5.7 0.6 0.5	10.7 10.0 2.7	45.0 19.4 6.4	25	0.2 0.02 0.02	0.4 0.4 0.11	1.8 0.8 0.3
DBP	Child 4-11y Adult	4.1 1.5	7.6 2.2	30.5 7.3	6.7	0.6 0.2	1.1 0.3	4.6 1.1
DIBP	Adult		1.5	4.2	1250		0.001	0.003
BBP	Child 4-11y Adult	0.42 0.22	0.77 0.88	4.5 4.0	500	0.001 0.0004	0.002 0.002	0.009 0.008
Biomonitoring total	**Child** **Adult**	**5.1** **2.2**	**18** **7.3**	**54** **22**		**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

The calculated exposure and risk based on biomonitoring data indicate that the risk is not controlled for some population groups. For the 95th percentiles the RCR are above 1 for both child and adults, while the RCR is above 1 for child based on high median exposure values. These results are supported by the results from Koch et al. (2011). Koch et al. (2011) have cumulated the daily exposure of phthalates to children in the age 5-6 years and compaired this exposure to a relative cumulative TDI. This shows that only very few children exceeded the individual TDI for the phthalates. Looking at the cumulative exposure of only the two phthalates DEHP and DBP, the cumulative TDI is exceeded for the 95 percentile and 7 of the 108 children have exceeded the cumulative TDI for these two phthalates alone. The exposure measured by Koch et al. (2011) gives RCR values of 0.46 for median values and 1.69 for the 95 percentile. This is comparable with the RCR based on the lowest median and the highest median in Table 32, respectively.

The biomonitoring data gives an indication of the level of exposure that some people have actually been exposed to. The RCR for biomonitoring can therefore be compared to the estimated RCR for articles, indoor environment and food.

B.10.1.6 Risk characterisation of combined exposure to the four phthalates

This section presents the results of combined RCR values from food, indoor environment, articles and biomonitoring data (Table 33). The RCR values are brought forward from section *B.10.1.5 Humans exposed to articles, food and indoor environment.* Here, RCR values for exposure from all sources are combined to a total RCR value for each age group. As there is only one RCR value from articles and indoor air present only one estimate of the mean exposures is used for the "lowest median" and "highest median" exposure scenarios. For biomonitoring data, food and dust "lowest median" is given in order to make a scenario representing the lower exposures. The combined RCR values can be interpreted as described in the introduction: RCR values above 1 indicate exposure levels above the derived no-effect levels (DNEL), and the risk is considered not to be controlled.

Table 33 RCR values for all four phthalates combining exposure from dust, food and consumer products. RCR values are from Table 29, Table 30, Table 31 and Table 32.

	Age	RCR for "lowest median" exposure estimates	RCR for "high median" exposure estimates	RCR for realistic worst case scenario (95^{th} percentiles or max)
RCR articles	**2-year old** **6/7-year old** **Adult**	0.066 0.74 0.19	0.066 0.74 0.19	0.18 7.65 1.62
RCR dust	**2-year old** **6/7-year old** **Adult**	0.11 0.072 0.009	0.35 0.23 0.032	1.63 1.08 0.14
RCR indoor air	**2-year old** **6/7-year old** **Adult**	0.01 0.012 0.002	0.01 0.012 0.002	0.05 0.06 0.01
RCR indoor environment	**2-year old** **6/7-year old** **Adult**	**0.12** **0.084** **0.011**	**0.36** **0.242** **0.034**	**1.68** **1.14** **0.15**
RCR articles and indoor enviroment	**2-year old** **6/7-year old** **Adult**	**0.186** **0.824** **0.201**	**0.426** **0.982** **0.224**	**1.86** **8.79** **1.77**
RCR food	**2-year old** **6/7-year old** **Adult**	0.24 0.17 0.078	2.2 0.97 0.33	4.24 2.13 1.82
RCR total (articles, indoor environment and food)	**2-year old** **6/7-year old** **Adult**	**0.43** **1.0** **0.28**	**2.7** **1.9** **0.55**	**6.10** **10.9** **3.59**
RCR biomonitoring	**Child** **Adult**	**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

The exposure data on food are, as mentioned, most probably overestimated as there are no precise data on the exposure from food today. It is therefore not possible to say how large the exposure from food is today, but an exposure from food will still take place because of phthalates in the environment. A study by Gärtner et al. (2009) shows that the exposure also still can take place from food contact material. Gärtner et al. (2009) have analysed the migration of phthalates in infant food packed in recycled paperboard, and this study shows that phthalates and especially DIBP can still be found in infant food collected in the beginning of 2009. The concentrations are though considered as low and the median concentration in the food of 20.3 ng/g. The Danish authorities have made market surveillance on phthalates in food contact materials and these market surveillance results show that some food contact materials exceeds the limit for phthalates.

The cumulated RCR from articles and indoor environment can be compared to the RCR based on biomonitoring data, and the difference between the cumulated RCR from articles and indoor environment and the RCR based on biomonitoring, will give an indication of the exposure from food. In the cases where the total RCR from articles, indoor environment and food is comparable

with the biomonitoring data it is indicated that the exposure from articles and indoor environment is underestimated as it is expected that the exposure from food is overestimated.

It should be emphasized that RCR values based on biomonitoring could be overestimated. The biomonitoring data are coming from the time before the legislation of phthalates in food contact materials entered into force in 2008, but after the voluntary agreement on the phasing out of phthalates in tubes for milk and foils for food. Furthermore, the data are from before the EU ban of some phthalates in toys and childcare articles from 2007 (REACH Regulation annex XVII). Before the EU ban of phthalates in 2007, an emergency ban on 6 phthalates in toys and childcare articles for children less than 3 years of age were already in force. Even though the REACH regulation entered into force after the measurements of the biomonitoring data, attention were already on phthalates at the time of the measurements and as described an emergency ban were already in force in EU before the biomonitoring measurements. The RCR values based on biomonitoring data might therefore be lower if measured today, but because of attention on these phthalates at the time of the measurements of the biomonitoring data, the overestimation may not be that significant.

B.10.1.6.1 RCR from "lowest median" exposure estimates

The cumulated RCR values for exposure to phthalates from indoor environment and articles are below 1 for all age groups in the case where the total RCR values are based on the lowest median exposure estimates (lowest values in Table 29 and Table 30). These RCR values are in line with the RCR values based on biomonitoring data. This indicate that there is no exposure from food, which seems to be unlikely, as it is expected that food contains a certain concentration of phthalates due to phthalates in the environment.

If the contribution from food is included, the RCR reaches 1 for 6/7-year olds. As mentioned earlier it is expected that the RCR values for food might be overestimated as these are based on older data and a legislation on phthalates in food contact materials has been introduced in EU since. Taking the overestimation of the exposure from food into account, this would probably result in a total RCR for 6/7-year olds below 1. Furthermore the RCR for articles for 6/7-year old contribute to the total RCR with 0.74 of which 0.63 are from erasers (mouthing an eraser 1 hour per day) (based on data from Danish EPA, 2007). Thus, for children the use of a single product (erasers) can increase the RCR to approximately 1. If it is assumed that the eraser is only mouthed 10 minutes per day the RCR contribution from erasers would be 0.1. Calculating the total RCR with this assumption would give a total RCR of 0.5 which is very close to the RCR for biomonitoring data of 0.6. This is though with the inclusion of the exposure from food which might be too high. This indicates that the exposure from articles or indoor environment could be higher than calculated, with the assumption that erasers are only mouthed 10 minutes per day. This is in line with the fact that a limited number of articles have been analyzed and exposure to phthalates can happen from other articles than those taken into account in the calculations in this dossier.

B.10.1.6.2 RCR from selected "high median" exposure estimates

Where the total RCR values are based on selected high median exposure estimates of the cumulated RCR for articles and indoor environment is below 1, while the total RCR, when including exposure from food exceeds 1 for 2-year olds and 6/7-year old; for 2-year olds the major part of the exposure is from food and dust. The RCR values for food is expected to be overestimated as these are based on older data and legislation on phthalates in food contact materials has been introduced in EU since. The main exposure might therefore not be from food

today, but there are no new data to confirm this or to give a picture of the exposure from food today.

For articles it can be seen that exposure from single articles as for example erasers contributes to a high exposure. For 6/7-year olds the exposure from DEHP from erasers alone gives a RCR value of 0.63 (mouthing an eraser 1 hour per day) and the total RCR from the four phthalates from articles are 0.74. If it is assumed that the eraser is only mouthed 10 minutes per day the RCR contribution from erasers would be 0.1. Calculating the total RCR with this assumption would give a total RCR of 1.41 which is very close to the RCR for biomonitoring data of 1.5. As the exposure of phthalates from food is expected to be overestimated, the comparison with biomonitoring data indicates that a larger exposure of phthalates will happen from articles and indoor environment.

RCR based on biomonitoring data indicate that the risk is not controlled for children. This is in line with the indications seen from the data from the exposure measurements form articles, indoor environment and food.

B.10.1.6.3 RCR for realistic worst case scenario

When using the high values (95^{th} percentiles or maximal exposure estimates) the cumulated RCR from indoor environment and articles exceeds 1 for all age groups. These RCR values are higher than the RCR values based on biomonitoring data even when the exposure from food is not added.

For 2-year olds, a slight increase in RCR from articles (compared to "high median" exposure) is due to exposure from sandals (contributing with an RCR of 0.2). For 6/7-year olds, the increase in RCR from articles (compared to "high median" exposure) is mainly due to phthalate exposure from erasers (intake of 8 mg eraser per day contributing with an RCR of 7) and from sandals (contributing with an RCR of 0.6). For adults, the increase in RCR from articles (compared to "high median" exposure) is mainly due to phthalate exposure from sandals (contributing with an RCR of 0.9) and sex toys (contributing with an RCR of 0.7).

There are uncertainties when these estimations of the risk from articles are made. It is for example assumed that the same adult person is using sex toys and the plastic sandal with the highest migration of phthalates, and that this person is using all the articles in the worst case use. This will overestimate the risk from articles especially in the worst case scenario. It could be expected that some persons use some articles as set up in the median exposure scenarios and some articles as set in the realistic worst case scenario, and the risk could be somewhere between these two scenarios.

Some articles will contain higher concentrations of phthalates than others and will therefore be responsible for a major contribution to human exposure to phthalates. The results show that for this scenario the total exposure from indoor environment and articles gives RCR above 1 for all age groups. Phthalates in dust and indoor air originates from phthalate containing articles in the homes. Single products or product applications may often be responsible for high exposure to phthalates. This makes it even more important to continue to try to reduce the contribution to human exposure to the four phthalates also for not yet identified sources.

The biomonitoring data gives RCR values above 1 for both children and adults indicating that the risk is not controlled. The estimated RCR values for articles, indoor environment and food give rise to even higher RCR values, which further indicates that the risk is not controlled for any groups of populations using realistic worst case scenarios.

B.10.1.7 Uncertainties in the RCR calculations

The exposure calculations are mainly based on data from chemical analysis of articles found on the Danish market. Chemical analyses are almost always encountered with uncertainties, which may result in both over- and underestimations. Many assumptions have been made and default values have been used in the calculations and this further contributes to the uncertainties in the calculations.

Biomonitoring data will though give a more precise estimation of the levels of exposure to phthalates that the population has been exposed to. The biomonitoring data found in the literature has been used in order to perform the calculations in this report, and these data show that certain groups of population will have an exposure that exceeds an acceptable level. These levels have been compared to the estimated levels of exposure, which are found to be in the same order of magnitude, indicating that the estimated levels of exposure are trustworthy.

B.10.2 Environment

Not relevant

B.11 Summary on hazard and risk

The four phthalates have a very widespread and dispersive use. They can be found in a great variety of articles from floor coverings to sandals. Due to this widespread use in many articles a concern is raised regarding human exposure to phthalates in articles. Individuals are exposed to phthalates through inhalation (phthalates emitted from wall paper, floor covering and other sources), ingestion (via e.g. food, toddlers suckling on plastic materials), mucous membranes and dermal exposure for their whole lifetime, since the intrauterine life.

The phthalates DEHP, DBP, BBP and DIBP were reported to affect testicular functions and to have adverse effects on sexual differentiation during the developmental process. They were furthermore found to exert anti-androgenic effects. These toxicological effects of the four phthalates have raised concerns regarding their endocrine-disrupting chemical properties in terms of reproductive and developmental disorders in humans.

Overall, the selected DNELs for these phthalates (DEHP 25 µg/kg bw/day ; DBP 6.7 µg/kg bw/day; BBP 500 µg/kg bw/day ; DIBP 1250 µg/kg bw/day) based on NOAELs for anti-androgenic effects in developmental studies are considered the most relevant available data for the current risk assessment, as dose addition is used to calculated the risk.

The exposure and risk is calculated for three population groups (2-year old, 6/7 year old and adults) for articles, indoor environment and food, respectively. To calculate the risk a RCR is calculated as a total RCR for all the four phthalates. If the RCR is above 1 the risk is not controlled. A RCR value is calculated for a worst case scenario/95th percentile, high median exposure and the lowest median exposure. The 95th percentile estimates are considered to be "realistic worst case exposure" levels. The "high median" exposure estimates were selected as high median value of exposure to each phthalate from each source. In order to make a scenario representing lower exposures, we selected the lowest median value of each phthalate from each source.

B.11.1 Exposure and risk from articles

In three projects from 2010 the Danish EPA has demonstrated that the four phthalates can be found in several articles. Mainly articles contributing to the exposure of children to phthalates, either via direct contact or indirectly via indoor air, have been brought into focus. The content and migrations of the four phthalates in the articles, to which children are directly exposed to as well as other articles, has been measured and used in the exposure assessment.

The migration from especially three types of articles resulted in extensive exposure to humans: sandals, sex toys and erasers.

It should be noted, that the high exposure from sandals to adults is due to a high migration of phthalate measured from a single pair of plastic sandals.

The use of sex toys can lead to a very high exposure as some sex toys have shown to contain and migrate very high concentrations of phthalates. The migration will to a high extent also depend on the use of an oil based lubricant. The migration of DEHP has shown to increase with a factor of more than 900 with the use of an oil based lubricant.

The possible intake of erasers has specifically been calculated for children in the early school-age (6/7- year olds). The calculation of exposure to phthalates from erasers (high estimate) was based on intake of 8 mg eraser per day (corresponding to the weight of 2-3 sesame seeds per day). The RCR from intake of eraser based on mouthing for 1 hour/day is calculated to be 0.63 for lowest and highest median exposure estimate. For realistic worst case level the scenario of eating 8 mg is chosen, giving a RCR of 7.

In Table 34 the calculated RCR values for median and realistic worst case exposure can be seen.

Table 34 The calculated RCR values for articles.

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95^{th} percentiles or max)**
RCR articles	**2-year old** **6/7-year old** **Adult**	0.066 0.74 0.19	0.066 0.74 0.19	0.18 7.65 1.62

RCR values for articles show that the risk is not controlled in worst case situations for 6/7-year olds and for adults. This picture is opposite to "the normal situation", where it could be expected that the highest risk is for the 2-years old. The opposite picture demonstrated here is because of the exposure from single articles giving a high exposure as the sandals and sex toys for adults and the erasers for 6/7-year olds.

There are uncertainties when these estimations of the risk from articles are made. It is for example assumed that the same person is using sex toys and the plastic sandal with the highest migration of phthalates, and that this person is using all the articles in the worst case use. This will overestimate the risk from articles, especially in the worst case scenario. It could be expected

that some persons use some articles as set up in the median exposure scenarios and some articles as set worst case scenario, and the risk could be somewhere between these two scenarios.

However, it should be noted that the specific articles included in these exposure estimates represent only a few of numerous sources of phthalate exposure from articles. For that reason, the cumulative exposure may easily reach levels of serious concern and a need of further control and limitation of the exposure to phthalates, especially for children.

B.11.2 Exposure and risk via indoor environment

Phthalates have the capacity to persist indoors for years after they are introduced, and even after the primary source is removed. Phthalates are not chemically bound to the polymer matrix of e.g. vinyl flooring, and slow emission from the articles to air or other media usually occurs during the entire use phase of the articles.

Exposure from the indoor environment can happen via indoor air or via dust. The exposure via air is calculated with three different approaches.

The indoor air levels of the phthalates were simulated by the use of data from furniture/materials marketed in Denmark. By applying the simulation it was found that DEHP is by far the dominating phthalate in indoor air. For that reason, a calculation has also been made by applying the method referred to in the EU Risk Assessment Report on DEHP (EU RAR, 2008). Finally, these levels have been compared to air levels of DEHP found in the literature.

The total concentration of phthalates in air (phthalate vapours and phthalates associated to particles) was calculated to be approximately 4.8 resp. 1 $\mu g/m^3$ in a realistic worst case resp. realistic scenario. Based on these levels, RCR is calculated (Table 35).

The exposure from dust is calculated based on literature data and RCR values are calculated and added to the RCR values for indoor air as shown in Table 35.

Table 35 RCR values for indoor environment.

	Age	**RCR for "lowest median" exposure estimates**	**RCR for "high median" exposure estimates**	**RCR for realistic worst case scenario (95th percentiles or max)**
RCR indoor air	**2-year old** **6/7-year old** **Adult**	0.01 0.012 0.002	0.01 0.012 0.002	0.05 0.06 0.01
RCR dust	**2-year old** **6/7-year old** **Adult**	0.11 0.072 0.009	0.35 0.23 0.032	1.63 1.08 0.14
RCR indoor environment (air and dust)	**2-year old** **6/7-year old** **Adult**	0.12 0.084 0.011	0.36 0.24 0.034	1.68 1.14 0.15

The RCR values for indoor environment shows that the risk in not controlled for the 95th percentiles for 2-year olds and 6/7-year olds with RCR values of 1.65 and 1.16 respectively. While the risk is under control for the median exposure (RCR: 0.03-0.36).

Phthalates in the indoor environment originates from the articles in the indoor environment (vinyl flooring, balance balls and electronic devices). The concentration will for example depend on:

- the concentration of phthalates contained in the articles
- the emission rate from the surface of the articles
- physical activities in a room (whirling dust around)
- ventilation rates
- room temperatrure
- the level and frequency of cleaning number and volume of sinks (sorptive reservoirs) in the room.

A reduction of the use of phthalates has effects on the indoor air concentration levels of phthalates as seen in e.g. the simulated scenarios. There is a redistribution of phthalates going on via the air from the sources to all other surfaces in a room, including human skin, clothes, etc. The content in air may have consequences for building up phthalate levels in the indoor environment on surfaces and dust which then further contribute to the indirect exposure.

Thus there are arguments for the control and limiting of the sources that generate concentrations of phthalates in the indoor environment.

B.11.3 Exposure and risk via food

The exposure of the four phthalates via food is calculated based on literature data. There are though only data on the levels of phthalates from around 1990. These data do not seem to take into account that a voluntary agreement of the phasing out of phthalates in tubes for milk and foils for food that were one of the large exposure pathways. Furthermore EU legislation on phthalates in food contact materials entered into force in 2008 and this is expected to lower the exposure of phthalates from food. A study by Gärtner et al. (2009) has analysed the migration of phthalates in infant food packed in recycled paperboard, and this study shows that phthalates and especially DIBP can still be found in infant food collected in the beginning of 2009. The concentrations are though considered as low and the median concentration in the food of 20.3 ng/g. The Danish authorities have made market surveillance on phthalates in food contact materials and these market surveillance results show that some food contact materials exceeds the limit for phthalates.

It can therefore still be expected that food will contain phthalates, due to the fact that one of the sources to phthalates in food is the environment, that some food contact materials will be able to migrate phthalates and market surveillance has shown that the limits are still exceeded from time to time. Phthalates in food from environmental pollution, can originate from the production of articles, articles used out door and from the articles ending as waste and finally in the environment. US EPA states, that there are limited data on the migration of phthalate from plasticized PVC into environmental media. However, due the total volume of plasticized PVC produced, it is possible that PVC or other polymer/polymericlike materials containing phthalates may be long-term and dispersive sources of human and environmental exposures to phthalates (US EPA 2009). There are no recent studies to confirm declining levels of the four phthalates in food today and the measurements of the exposure of the four phthalates in food are therefore based on the available data. The calculated exposure of the four phthalates from food will therefore most probably be overestimated. The data used are data from opinions from EFSA (2005) on DEHP, DBP and BBP for use in food contact materials and these data are based on phthalate intake from food from two Danish studies, based on dietary measurements.

Table 36 below shows the RCR values calculated from exposure via food for the four phthalates.

Table 36 RCR values for food.

	Age	RCR for "lowest median" exposure estimates	RCR for "high median" exposure estimates	RCR for realistic worst case scenario (95th percentiles or max)
RCR food	**2-year old** **6/7-year old** **Adult**	0.24 0.17 0.078	2.2 0.97 0.33	4.24 2.13 1.82

Based on the available data on food it may be concluded, that these results strongly indicate the need for further control and limitation of the exposure to phthalates, especially for 2-year olds, as the RCR is above one from the exposure from food alone, based on "high median" RCR (RCR 2.2). RCR for 6/7-year olds from food alone is very close to 1 (0.97) for "high median". It should though be emphasized that these calculations are based on old data and that the exposure must be expected to have been decreased. There are though no available data that indicate the level of exposure from food today.

B.11.4 Exposure and risk based on biomonitoring data

The main part of the studies are performed on adult humans, a few studies are made on children older than 6 years and only one study presents data on 2 to 4 year olds. The biomonitoring data are measured before the legislation of phthalates in food contact materials entered into force in 2008, but after the voluntary agreement on the phasing out of phthalates in tubes for milk and foils for food. The data are from before the EU ban of some phthalates in toys and childcare articles from 2007 (REACH Regulation annex XVII). Before the EU ban of phthalates in 2007, an emergency ban on 6 phthalates in toys and childcare articles for children less than 3 years of age were in force. Even though legislation has entered into force after the reporting of the biomonitoring data, the phthalates where already in focus at the time the monitoring were performed. The RCR values based on biomonitoring data might therefore be lower if the monitoring were performed today.

Table 37 below shows RCR values on biomonitoring.

Table 37 RCRs based on biomonitoring data

	Age	RCR for "lowest median" exposure estimates	RCR for "high median" exposure estimates	RCR for realistic worst case scenario (95th percentiles or max)
RCR biomonitoring	**Child** **Adult**	**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

As can be seen from the results in Table 37 of biomonitoring data it is only the RCRs based on the lowest median, that where lower than 1 (0.6) for children. Even RCRs based on high median are inclining that there is a risk that is not sufficiently controlled

B.11.5 Cumulative risk assessment

It is not possible to give a precise estimation of the level of exposure of the four phthalates from food, as the data are old and the concentrations of the four phthalates are assumed to have decreased since the origin of the data.

In Table 38 below the sum of the RCR values can be seen and compared to the RCR values based on biomonitoring data.

Table 38 Total RCR values and RCR values based on biomonitoring data.

	Age	RCR for "lowest median" exposure estimates	RCR for "high median" exposure estimates	RCR for realistic worst case scenario (95^{th} percentiles or max)
RCR indoor environment and articles	**2-year old** **6/7-year old** **Adult**	**0.19** **0.82** **0.20**	**0.43** **0.98** **0.22**	**1.86** **8.79** **1.77**
RCR total	**2-year old** **6/7-year old** **Adult**	**0.43** **1.0** **0.28**	**2.7** **1.9** **0.55**	**6.10** **10,9** **3.59**
RCR biomonitoring	**Child** **Adult**	**0.6** **0.2**	**1.5** **0.4**	**5.4** **1.4**

The sum of the median RCR values for articles and indoor environment are lower than the RCR values based on biomonitoring data for all three age groups. This indicates that the difference between these is from the exposure from food. If the same comparison is made for the 95^{th} percentiles, then the sum of the RCR values for articles and indoor environment are higher than the RCR values based on biomonitoring data. This could be because of an overestimation of the exposure from articles in the worst case scenario as it is assumed that one person is using more than one article in a worst case use.

Comparing and adding the RCR values for the articles, indoor environment and food strongly indicate that the exposure of the four phthalates may constitute a risk for anti-androgenic effects among exposed individuals following cumulative exposure to the four phthalates for these age groups. Even though they have different potency their similar mode of action makes it reasonable to perform dose addition (see B.1.4.2) to predict the overall exposure and the combination effects of these substances. In a worst case scenario the total RCR values indicate a risk to all population groups and even though the total calculated RCR values (articles, indoor environment and food) seem to be overestimated especially for the articles and food, the RCR for biomonitoring data also show values above 1 for the 95^{th} percentile.

The cumulative approach to this risk assessment makes it clear that even with relatively low background phthalate exposures (such as the "low median" values for children) giving an RCR below 1, an added exposure from articles to any one of the four phthalates in sufficiently high amounts may result in an increase of the RCR above 1 and thus, identify an increase in the risk for anti-androgenic effects among exposed individuals.

Phthalates in the indoor environment and in food have been estimated to be a significant source to the exposure of phthalates even though there are uncertainties in the estimations and the

assumptions made. Phthalates in the indoor environment are originating from the articles present indoor, as these articles will emit phthalates to the air and dust in the indoor environment. Phthalates found in food are originating from environmental pollution. This environmental pollution can come from articles used outdoor, the production of articles or when the articles end up as waste and finally in the environment. The main sources to the exposure of phthalates are therefore from the articles used and the articles having a direct contact to skin or mucous membranes.

It should be noted that there are uncertainties associated with both DNELs and exposure estimates and therefore, RCR values just above 1 should be interpreted bearing these uncertainties in mind. However, the approach using “low median”, “high median” and “realistic worst case” scenarios for the RCR calculations is an attempt to investigate the consequences of the uncertainty in exposure assessment by evaluating how much the total RCR changes when background exposure estimates change. RCR values just below 1 should also be interpreted cautiously because the RCR values only include considerations of the four specific phthalates. Thus cumulative risk assessment including also other anti-androgenic compounds than the four phthalates may therefore lead to even higher RCR values.

The data shows that for a large part of the population the risk is not sufficiently controlled and the exposure to DEHP, DBP, BBP and DIBP should be reduced.

B.11.6 Conclusion on hazard and risk

Risk Characterisation Ratio (RCR) values for “High median” and “realistic worst case” background exposure levels exceeding 1, is interpreted as an indication that the risk is not sufficiently controlled. It should be noted, however, that some of the exposure data from food, dust and the biomonitoring data are based on measurements performed before the restrictions on the use of these four phthalates in toys and certain childcare articles were implemented in the EU. On the other hand, it should also be underlined that attention was already paid to the four phthalates due to the temporary ban in toys and childcare articles that had been in place for several years.

The risk associated with high exposure of humans to anti-androgenic chemicals is thus a risk of impaired development of androgen-dependent organs. Pregnant women exposed to high levels of phthalates may have increased risk of having sons with malformations of the genitals (hypospadias and cryptorchidism), low sperm count and increased risk of testis cancer, as proposed in the hypothesis of the Testicular Dysgenesis Syndrome (TDS) (Sharpe & Skakkebaek, 2008). In line with this, it may be hypothesized that attempts to decrease internal exposure to these phthalates and other anti-androgenic chemicals may decrease the incidence of TDS in humans.

It should be noted that the specific articles included in the exposure estimates carried out in this dossier represent only a few of numerous sources of phthalate exposure from articles. Single articles or article applications may often be responsible for high exposure to phthalates and other anti-androgenic chemicals. This makes it even more important to continue to try to reduce the contribution to human exposure also for not yet identified sources.

The data shows that for a large part of the population the risk is not sufficiently controlled and the exposure to DEHP, DBP, BBP and DIBP should be reduced. There are many possible sources to phthalates as environmental pollution ending up in the food. Amongst others, from the production of articles, during use of articles or when the articles end up in the waste stream.

The major contributors to the concentration of the four phthalates found in the indoor environment in the gas phase, particles in the air and dust are the articles like e.g. furniture, toys, PVC flooring and wall paper.

Phthalates in the indoor environment and in food have been estimated to be a significant source to the exposure of phthalates even though there are uncertainties in the estimations and the assumptions made. Phthalates in the indoor environment are originating from the articles present indoor, as these articles will emit phthalates to the air and dust in the indoor environment. The main sources to the exposure of phthalates are therefore from the articles used and the articles having a direct contact to skin or mucous membranes. A regulation of the content of DEHP, DBP, BBP and DIBP in articles intended for indoor environment, and articles where a direct contact to the skin or mucous membranes may be expected, would contribute to the reduction of the human exposure to these phthalates to an acceptable level.

C. Available information on alternatives

The decrease in production volumes in recent years reflects the fact that DEHP for many applications has been replaced by other substances, primarily di-isononyl phthalate (DINP). Below is given a review of the most important and used alternatives that have been mentioned in section B of this dossier.

Non-phthalate alternatives have mainly been applied by industry / importers for applications where there has been a general and public concern to human exposure to the substance: toys, medical products and food packaging are examples. Applications for which the selected alternatives are specifically mentioned by suppliers of the alternatives are shown in Table 10 n section B.2.2.13, but the substances may probably be used for other applications as well.

It is evident from the data reviewed that there is a wide variability in the level of information available (and validity of data sources) amongst the potential alternatives and, as such, drawing definitive conclusions on whether any additional risks for the environment would be introduced if these were to be substituted for DEHP is not straightforward for all substances.

Besides the replacement of the four phthalates with other plasticisers, the soft PVC itself may be replaced with a range of alternatives such as e.g. polyurethane (PU). Generally speaking it seems that many of the materials seem to have equal or better environmental, health and safety, performance and cost profiles, but clear conclusions are complicated by the fact that it is difficult to include all aspects of the materials' lifecycles and potential uses. Other materials are therefore not included in this dossier; however, after the chapters on the alternative plasticiers there is a short chapter discussing very briefly some of the alternative flexible polymers.
Some of the alternative plasticisers are also not included in this dossier. This is the case for DIDP, DIHP, DIOP, DPHP, TOTM, DOA, ESBO, Isodecyl benzoate and DOTH. The reason is basically lack of sufficient data for evaluating the substances or that the substance is classified (TOTM) and also the fact that these substances generally are used only in rare cases and can be substituted with other alternatives.

The widespread use of the alternatives is indicated in the previous section B and the human health and environmental related issues of the most important alternatives is given below.

C.1 Identification of potential alternative substances and techniques

Alternatives for the four phthalates could be both other ortho-phthalates, non-phthalate plasticisers and other polymeric materials than PVC, which do not need to be plasticised. The suitability of the alternatives will be dependent on the final article and one alternative might be suitable for one article and not for other articles, but suitable alternative plasticisers have been identified for all applications of the four phthalates.

Most of the work on alternatives to the four classified phthalates has in the past been carried out with respect to toys and childcare articles. However, it has to be underlined that the results from these investigations are general and comply to most if not all other uses in

articles as such. Some of the text and information in this chapter is therefore related to toys but it can generally be concluded, that the information is also valid for articles as such.

DIBP

It is stated in the registration dossiers by the importers/producers that "DNELs for the general population are not required - no consumer exposure". On the webpage DIBP Information Centre (http://www.dibp-facts.com), which is an initiative of the European Council for Plasticisers and Intermediates (ECPI) it is however stated that: "*Diisobutyl phthalate (DIBP) is a specialist plasticiser often used in combination with other high molecular weight phthalates. It is a fast fusing plasticiser which by itself it is too volatile for PVC applications. It is frequently used as a gelling aid in combination with other plasticisers. [It is] A plasticiser for nitrocellulose, cellulose ether, and polyacrylate and polyacetate dispersions.*"

This is somewhat contra dictionary and it is assumed in this restriction proposal that the statements given by ECPI are correct.

It is also stated on the DIBP Information Centre's webpage that" *Diisobutyl phthalate (DIBP) has very similar application properties to Di-n-butyl phthalate (DBP) and may therefore be used to substitute for DBP in most, if not all, of its applications. These range from the plasticisation of PVC to the production of paints, printing inks and adhesives.*"

From this it is obvious that the alternatives to DBP can be used as substitutes for DIBP as well and this is assumed throughout this restriction proposal.

Alternatives to the six phthalates DEHP, DBP, BBP, DINP, DIDP and DNOP have been used in toys since the EU ban in 2007. Table 39 below lists plasticisers used in toys and childcare articles in which the phthalates DEHP, DBP and BBP are restricted in concentrations above 0.1 %. If the toys and childcare articles can be placed in the mouth DINP, DIDP and DNOP are also restricted in concentrations above 0.1 %. The substances reported by Danish manufacturers have specifically been used by the manufacturers as substitutes for phthalates, whereas some of the plasticisers reported in the surveys of plasticisers in toys and childcare products from other EU countries may in fact not have substituted a former use of phthalates (Maag et al., 2010).

Table 39 Plasticisers found in toys and childcare articles (Maag et al., 2010)

Group of plasticiser	Chemical name	Abbreviation	CAS no.	Occurrence in toys and childcare articles		
				Reported by Danish manufacturers /suppliers *2	Survey in the Netherlands 2007, % of samples	Survey in Germany, Austria and Switzerland 2007, % of samples

Group of plasticiser	Chemical name	Abbreviation	CAS no.	Occurrence in toys and childcare articles		
				Reported by Danish manufacturers /suppliers *2	Survey in the Netherlands 2007, % of samples	Survey in Germany, Austria and Switzerland 2007, % of samples
Phthalates	Diisononyl phthalate	DINP	28553-12-0 68515-48-0	Only non-phthalates reported	49%	10%
	Diisodecyl phthalate	DIDP	26761-40-0 271-091-4	_"_	15%	2%
	Diisobutyl phthalate	DIBP	84-69-5	_"_	2%	2%
Cyclohexanes	Di-isononyl-cyclohexane-1,2dicarboxylate	DINCH	166412-78-8	X	25%	48%
Terephthalates	Di (2-ethyl-hexyl) terephthalate	DEHT, DOTP	6422-86-2	X	7%	10%
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6	X	*3	*3
Other alkyl esters	Trimethyl pentanyl diisobutyrate	TXIB	6846-50-0		14%	11%
Citrates	Acetyl tributyl citrate	ATBC	77-90-7	X	9%	10%
Aliphatic dibasic esters	Diisononyl adipate	DINA	33703-08-1	x	6%	4%
	Bis(2-ethylhexyl) adipate	DEHA	103-23-1		4%	2%
	Diisobutyl adipate	DiBA	141-04-8		0.6%	
	Dioctyl sebacate	DEHS	122-62-3		0.6%	
Mixed alkyl aryl esters	Mixed diesters neopentylglycol-benzoate/2-ethylhexanoate	NPG-EHA-BA				7%
	Mixed triesters 1,1,1-trimethylol-propane-benzoate/2-ethylhexanoate	TPG-EHA-BA				2%
	Hexanoic acid, 2-ethyl, mixed triesters with benzoic acid and trimethylopropane	LG-flex BET	610787-76-3	x	*3	*3
Polyesters	Polyadipate	PA				3%
Epoxy esters and epoxidized oils	Epoxidized soy bean oil	ESBO	8013-07-8			1%

Group of plasticiser	Chemical name	Abbreviation	CAS no.	Occurrence in toys and childcare articles		
				Reported by Danish manufacturers /suppliers *2	Survey in the Netherlands 2007, % of samples	Survey in Germany, Austria and Switzerland 2007, % of samples
Alkyl acetyl esters	Tert-butyl acetate *1	TBAC	540-88-5		11%	
Alkylphenols	Nonylphenol *1		25154-52-3		18%	
Trimellates	Tri-(2-ethylhexyl)-trimellitate	TEHTM (TOTM)	3319-31-1			1%

*1These substances are usually not mentioned as plasticisers in plastics, but may serve other purposes in the plastics. It has not been confirmed that the substances are actually used as plasticiser in the plastics.

*2 "X" indicates that the substance is reported by all manufactures. "x" indicates that the substance is reported by one manufacturer only.

*3 It is not clear from the report whether the surveys have screened for these alternatives.

Three non-phthalate plasticisers were found in a significant percentage of the samples and are reported by all responding Danish manufacturers of toys as used as alternatives to phthalates: DINCH, DEHT and ATBC. All three are marketed as general plasticiser alternatives to DEHP (Maag et al., 2010). Two of these three plasticisers were also found in a new study from the Netherlands. They identified DINCH, DEHT and TXIB (Jansen & Bremmer, 2009). Among the non-phthalate plasticisers there does not seem to be any plasticisers that can make a one-to-one substitution. Which substitutes are suitable depends on the actual processing conditions and the desired properties of the final product. Finding the right plasticiser for a given application is often a complex process, as many technical criteria have to be met simultaneously. Comprehensive testing of the performance of the polymer/plasticiser system is often required. By way of example one Danish manufacturer reports that the development led to the use of a mixture of ATBC, DINCH and DEHT, which could be blended in a variety of combinations to achieve softened PVC that performed to the required standards with the existing production setup (Maag et al., 2010).

In view of the wide spectre of alternative plasticisers available Magg et al. (2010) contacted producers of plasticisers directly in order to get more detailed information on the experience gained on the market with alternative plasticisers. The questions were on the following issues:

- Proposals for alternative plasticisers for specified traditional DEHP, DBP and BBP applications;
- Level of experience gained on the market with proposed alternatives, according to the manufacturers' own judgement (4 simplified categories);
- Important processing adjustments, if any, compared to DEHP, DBP and BBP, respectively;
- Limitations in use of alternatives for specified applications;
- Prices of alternatives.

Table 40 below lists the identified plasticisers and indicates the plasticisers selected for further evaluation in this study (marked in gray).

Table 40 Identified plasticisers and reason for selection for environmental and health assessment

Group of plasticiser	Chemical name	Abbre-viation	CAS no.	Occurrence in toys and childcare articles			Proposed by manufacturers of plasticisers as alternatives for: *2,3	Previously evaluated by *4	Reason for selection
				Reported by Danish manufacturers /suppliers *7	Survey in the Netherlands 2007,% of samples,	Survey in Germany, Austria and Switzerland 2007,% of samples,			

Group of plasticiser	Chemical name	Abbre-viation	CAS no.	Occurrence in toys and childcare articles			Proposed by manufacturers of plasticisers as alternatives for: *2,3	Previously evaluated by *4	Reason for selection
				Reported by Danish manufacturers /suppliers *7	Survey in the Netherlands 2007,% of samples,	Survey in Germany, Austria and Switzerland 2007,% of samples,			
Phthalates	Diisononyl phthalate	DINP	28553-12-0 68515-48-0	Only non-phthalates reported	49%	10%	DEHP *6	E	
	Diisodecyl phthalate	DIDP	26761-40-0 271-091-4	_"_	15%	2%	DEHP *6		
	Diisoheptyl phthalate	DIHP	71888-89-6	_"_			DEHP/DBB blends *6		
	Diisodecyl phthalate	DIBP	84-69-5	_"_	2%	2%	DBP *6	E	
Benzoates	Diethylene glycol dibenzoate	DEGD	120-55-8				DEHP, BBP, DBP (M)		Significant market experience as alternatives for some BBP applications, according to producer. Indicated as important substitute for BBP and DBP in ECHA study
	Dipropylene glycol dibenzoate	DGD	27138-31-4				DEHP, BBP, DBP	E,C,T,K	Significant market experience as alternatives for some BBP applications, according to producer. Indicated as important substitute for BBP and DBP in ECHA study
	Triethylene glycol dibenzoate	TGD	120-56-9				DEHP, BBP, DBP (M)		
Cyclohexanes	Di-isononyl-cyclohexane-1,2dicarboxylate	DINCH	166412-78-8	X	25%	48%		E,T,S	Most used alternative in toys, according to surveys
Terephthalates	Di (2-ethyl-hexyl) terephthalate	DEHT, DOTP	6422-86-2	X	7%	10%	DEHP	E, T, S	Significant market experience as alternatives for some applications, according to producer. Much used alternative in toys, according to surveys
	Di-butyl terephthalate	DBT	1962-75-0				DBP, BBP		
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6	x			DEHP, BBP, DBP	E,N	Significant market experience as alternatives for some applications, according to producer.

Group of plasticiser	Chemical name	Abbre-viation	CAS no.	Occurrence in toys and childcare articles			Proposed by manufacturers of plasticisers as alternatives for: *2,3	Previously evaluated by *4	Reason for selection
				Reported by Danish manufacturers /suppliers *7	Survey in the Netherlands 2007,% of samples,	Survey in Germany, Austria and Switzerland 2007,% of samples,			
Glycorol acetyl esters	Glycerol Triacetate	GTA	102-76-1				BBP, DBP	E	Significant market experience among alternatives for some applications, according to producer.
Other alkyl esters	Trimethyl pentanyl diisobutyrate	TXIB	6846-50-0		14%	11%		C	Frequently used alternative in toys, according to surveys
Citrates	Acetyl tributyl citrate	ATBC	77-90-7	X	9%	10%	DEHP, BBP, DBP	C,P,K,S	Much used alternative in toys, according to toy studies; significant market experince among alternatives for some applications, according to producer
Aliphatic dibasic esters	Diisononyl adipate	DINA	33703-08-1	x	6%	4%			Frequently used alternative in toys, according to surveys; adipate representative of DEHP substitutes
	Dibutyl adipate	DBA	105-99-7				DBP		
	Bis(2-ethylhexyl) adipate	DEHA	103-23-1		4%	2%		C,P,K,T,S	Reported by SCENIHR(2008) to have reproductive toxicity
	Benzyl octyl adipate	BOA	58394-64-2				DEHP		
	Diisobutyl adipate	DiBA	141-04-8		0.6%				
	Dioctyl sebacate	DEHS	122-62-3		0.6%			C,K	
Mixed alkyl aryl esters	Mixed diesters neopentylglycol-benzoate/2-ethylhexanoate	NPG-EHA-BA				7%			
	Mixed triesters 1,1,1-trimethylol-propane-benzoate/2-ethylhexanoate	TPG-EHA-BA				2%			
	Hexanoic acid, 2-ethyl, mixed triesters with benzoic acid and trimethylopropane	LG-flex BET	610787-76-3	x					
Polyesters	Polyadipate	PA				3%		C	

Group of plasticiser	Chemical name	Abbre-viation	CAS no.	Occurrence in toys and childcare articles			Proposed by manufacturers of plasticisers as alternatives for: *2,3	Previously evaluated by *4	Reason for selection
				Reported by Danish manufacturers /suppliers *7	Survey in the Netherlands 2007,% of samples,	Survey in Germany, Austria and Switzerland 2007,% of samples,			
Castor oil derivatives	12-(Acetoxy)-stearic acid, 2,3-bis(acetoxy)propyl ester)	COMGHA 1	330198-91-9				DEHP, BBP, DBP	S *5	Significant market experience, expected low toxicity (food ingredient)
	Octadecanoic acid, 2,3-(bis(acetoxy)propyl ester.	COMGHA 2	33599-07-4				DEHP, BBP, DBP	S *5	
Epoxy esters and epoxi-dized oils	Epoxidized soy bean oil	ESBO	8013-07-8			1%		S, K	
Alkyl acetyl esters	Mixture of glycerine acetates (Unimoll AGF)		-				DEHP		
	Tert-butyl acetate *1	TBAC	540-88-5		11%				
Alkylphenols	Nonylphenol *1		25154-52-3		18%				
Glycerols	Trimethylolpropane (hexaglycerine) *1	TMP	77-99-6	x					
Trimellates	Tri-(2-ethylhexyl)-trimellitate	TEHTM (TOTM)	3319-31-1			1%		S, T,K,S	Reported by SCENIHR(2008) to have reproductive toxicity

- *1 These substances are usually not mentioned as plasticisers in plastics. It has not been confirmed that the substances are actually used as plasticiser in the plastics.
- *2 (M): In mixtures with other substances.
- *3 As proposed for this study. Information has not been obtained for all substances. An empty cell in this column does not necessary mean that the substances are not suitable substitutes.
- *4: Sources: E: ECHA, 2009 (a, b, c); C: Stuer-Lauridsen et al., 2001;T: TURI, 2006; K: Karbæk, 2003; P: Postle et al., 2000; S: SCENIHR, 2008. N: Nilsson et al., 2002.
- *5 The evaluated CAS No is "acetylated monoglycerides of fully hydrogenated castor oil", CAS No 736150-63-3 which consist of a mixture of the above mentioned substances.
- *6 As recommended at manufacturers' web pages.
- *7 "X" indicates that the substance is reported by all manufactures. "x" indicates that the substance is reported by one manufacturer only.

The selection was based on the following factors:

- Experience with the substances on the market, based on data collected from suppliers of alternative plasticisers.
- Occurrence as plasticisers in toys and childcare articles, based on the studies of plasticisers in these products described above.
- The list should cover the main alternatives for each of the three phthalates DEHP, DBP, and BBP.
- Representation of major substance groups used as plasticisers based on description above.
- Substances with identified significant environment and health effects (CMR or PBT, based on reviews in ECHA, 2009a) were not suggested for further assessment.

C.2 Assessment of diisononyl phthalate (DINP)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Phthalate	1,2-Benzenedicarboxylic acid, di-C8-10 branched alkylesters, C9 rich	DINP	68515-48-0 28553-12-0

DINP is not a pure substance, but a complex mixture containing mainly C_9-branched isomers, with mean formula $C_{26}H_{42}O_4$ and mean molecular weight M=20.6 g/mol. DINP is not classified according to the CLP Regulation.

C.2.1 Availability of DINP

DINP is a widely used phthalate and is now used as an alternative to many applications where DEHP were used earlier. DINP is regulated in toys and childcare articles that can be put in the mouth by children, but not in all toys and childcare articles as is the case for DEHP, DBP and BBP. DINP can therefore still be used in certain kinds of toys and childcare articles.

DINP has a wide range of indoor and outdoor applications. 95 % of DINP is used as a plasticiser for flexible PVC used for construction and industrial applications, and durable goods (wire and cable, film and sheet, flooring, industrial hoses and tubing, footwear, toys, food contact plastics). The other five per cent is used in non-PVC applications (e.g., rubbers, adhesives, sealants, paints and lacquers, lubricants) (DINP-facts, 2011).

C.2.2 Human health risks related to DINP

DINP has been risk assessed in EU and most of the data on human health are from the EU risk assessment report from 2003. ECHA has made an evaluation of DINP in 2010, results from this evaluation is also used.

C.2.2.1 Toxicokinetic DINP

DINP is rapidly eliminated, less than 0.1% of the radioactivity was recovered in tissues after 72 hours. By oral and dermal routes, excretion is shared between urine and faeces. By dermal exposure, biliary excretion is shown (EU RAR, 2003).

C.2.2.2 Acute toxicity of DINP

It can be considered that DINP has a low acute oral, dermal and inhalation toxicity. No LD50/LC50 was reported from acute exposure by those routes of exposure (EU RAR, 2003).

C.2.2.3 Irritation

On the whole, DINP may be considered as a very slight skin and eyes irritant, with effects reversible in short time. Thus no classification is indicated according to the EU criteria for those different end points (EU RAR, 2003).

C.2.2.4 Sensitisation

In a mouse study, referenced in the ECHA evaluation of DINP (2010) the results suggest that both DEHP and DINP enhance allergenic responses by enhancement of IL-4 production in CD4+ T cells via stimulation of NF-AT-binding activity. The new studies give some evidence of a sensitizing potential of DINP, but the studies would need an in-depth assessment to evaluate the reliability and relevance to conclude on whether or not the results would lead to a different onclusion compared to the one drawn in the EU RAR (ECHA, 2010)

C.2.2.5 Repeated dose toxicity

The following paragraphs is from the ECHA evaluation (2010). The liver has previously been identified as the target organ for DINP effects. In the EU RAR several studies were included indicating that DINP acts as a peroxisome proliferator (PP), a mechanism which is considered to be of low or no relevance to humans. The PP effects of DINP have been tested in monkeys and no effects were seen which further supports this. In the EU RAR a NOAEL of 88 mg/kg bw/day was determined, based on a well-conducted chronic/carcinogenicity study in rats. The NOAEL was based on hepatic biochemical changes (increased ALT, AST), liver weight increase in both sexes together with histopathological findings (not related to specific PP effects).

During the review information was found that confirms the liver as the target organ for effects of DINP, and it has been further shown in mouse studies that DINP works through a PP mechanism (Kaufmann *et al*, 2002; Valles *et al*, 2003).

In a report from the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001) a lower NOAEL of 15 mg/kg bw/day based on findings of spongiosis hepatis in rats was determined. The reference on which this NOAEL is based was included in the EU RAR, but there is a difference in interpretation of spongiosis hepatis between the EU RAR and the CSTEE report and hence, this leads to differences in determination of the NOAEL. This is also mentioned in the communication from the Commission to the European Parliament on the restriction of the phthalates (http://eur-lex.europa.eu, CELEX: 52005PC0143). In this communication it is said that the CSTEE concluded that there is a need for limiting the risks based on this finding, while in the EU RAR it was concluded that there is no need for further information/testing or for risk reduction measures beyond those being already applied. ECPI does not support the lower NOAEL concluded by the CSTEE, but instead agrees on the NOAEL from the EU RAR. Their argument for this is presented in their “Review of Recent Scientific Data on Di-isononyl Phthalate (DINP) and Risk Characterisation for its use in Toys and Childcare articles” (ECPI, 2009), where it is *i.a.* stated that spongiosis hepatis is considered to be degenerative change in ageing rats, with no known counterpart in humans. It is also stated that the change seems to be male specific. An in-depth evaluation of the relevant information is needed before a firm conclusion on the NOAEL for liver effects can be determined.

C.2.2.6 Mutagenicity
In the EU RAR (2003), the conclusion was that DINP is not mutagenic *in vitro*, based on bacterial mutation assays or mammalian gene mutation assay, nor clastogenic in one *in vitro* cytogenicity assay and one *in vivo* assay on bone marrow cells. The conclusion in the EU RAR (2003) was that the findings in the rat studies on carcinogenicity were not relevant to humans.

C.2.2.7 Toxicity to reproduction
The following paragraphs are from the ECHA evaluation (2010). In adult rats, some effects were reported in the EU RAR, e.g. increased and decreased absolute and relative testis weight, but adverse effects were not histologically confirmed. The NOAEL for 276 mg/kg bw/day was assumed based on testicular effects. Based on the studies evaluated in the EU RAR, it was concluded that DINP does not cause any adverse effects on fertility.

In the EU RAR, several studies evaluating the developmental toxicity of DINP were reported. A decrease in live birth and survival indices was observed in a one-generation study, but not in a two-generation study in rats. A NOAEL of 622 mg/kg bw/day was determined based on the decrease in life birth and survival indices. In developmental studies, visceral and skeletal variations in the absence of maternal toxicity, or together with only slight maternal toxicity, were significantly increased and the NOAEL was determined to be 500 mg/kg bw/day. A decrease in mean offspring bodyweight was observed in one- and two-generation studies at the lowest dose tested and the LOAEL was estimated to be 159 mg/kg bw/day, the lowest value of the maternal dose range post-partum.

During the review new information on developmental toxicity was found. In one study (Borch *et al*, 2004), 750 mg/kg bw/day of DINP caused a reduction in testosterone content and production in testes of male rat foetuses on GD 21, but no statistical significant change in plasma testosterone levels was found.

In the rat study by Lee *et al* (2006) a small but significant decrease in neonatal foetal bw as well as a decreased anogenital distance (AGD; PND 1) was seen in male offspring at all doses tested also after bodyweight correction, and an increase in AGD was seen in females, but only at the highest dose tested. There was no dose-dependent change in serum estradiol or testosterone levels (PND 7). In another rat study (Masutomi *et al*, 2003) some significant effects on organ weights (adrenals, uterus, brain) were seen in offspring after *in utero* and postnatal exposure to DINP, but only at the highest dose tested (20.000 ppm, corresponding to a maternal intake of approx. 1165 mg/kg bw/day during gestation, and approx. 2646 mg/kg bw/day during lactation). However, the changes were not consistent when comparing absolute and relative weights. At the same dose toxic effects on both dams and offspring were seen. There was also a decreased body weight gain in offspring (both sexes), as well as a decrease in testes weight, both absolute and relative. The decrease in testes weight could indicate an anti-androgenic action of DINP, but the dose level where it was seen was high, and the effect seemed to be transient as there was no significant change at final necropsy. A change in degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells were observed in the testes. The changes were only slight/minimal, but were present in 80% of the animals. There were no effects on puberty onset or on oestrous cyclicity. In another study in rats by the same authors (Masutomi *et al*, 2004), no effects on offspring after maternal exposure to DINP were seen.

In the ECPI review on DINP, the validity of some of the studies is questioned. The NOAELs in most of these studies are higher than the ones determined in the EU RAR.

In the study by Lee *et al* (2006) no calculation of doses in mg/kg bw/day had been done, but when comparing the doses with other rat studies, a dose of 40 ppm would correspond to

approximately 2 mg/kg bw/day. The results of this study is not considered enough to justify a change in the NOAEL for developmental toxicity determined in the EU RAR and can only be used as additional information. However, the results should be assessed in conjunction with the other studies indicating a potential endocrine disrupting (ED) effect of DINP and with the studies included in the EU RAR.

In vitro tests reported in the EU RAR did not reveal any estrogenic activity of DINP. Neither any reproducible, dose-dependent estrogenic effects were seen in the uterotrophic assay/vaginal cell cornification assay. Regarding anti-/androgenic activities, there was one *in vivo* study in rats (Gray *et al*, 2000) reported in which males with areolas were observed, but no details on incidence were given. There were also indications of anti-androgenic effects seen and the DINP treatment group was reported to have malformations of testis, epididymis, accessory reproductive organs and external genitalia.

During the review several new studies evaluating potential effects of DINP on the endocrine system were reported. Masutomi *et al* (2003) studied the effects of DINP exposure in rats (400, 4.000 and 20.000 ppm) and concluded that the highest dose tested caused degeneration of meiotic spermatocytes and Sertoli cells in the testis and decrease of corpora lutea in the ovary at week 11, although changes remained minimal or slight. In another study with a similar exposure (Masutomi *et al*, 2004), no effects of DINP on luteinising hormone (LH), follicle stimulating hormone (FSH) or prolactin levels were found. In an *in vivo* study (Hershberger assay in castrated male SD rats; Lee & Koo, 2007) there are indications that DINP may cause anti-androgenic effects. A decrease in seminal vesicle weight was seen at 100 and 500 mg/kg /d, and a decrease in levator ani/bulbocavernosus muscles (LABC) weights at 500 mg/kg bw/day. One new study (Main *et al*, 2006) was found where they studied whether phthalate monoester contamination of human breast milk had any influence on the postnatal surge of reproductive hormones in newborn boys as a sign of testicular dysgenesis. Mono-isononyl phthalate was found in the highest concentration of all phthalate monoesters included in the study. In the study a correlation was found between the metabolite MINP and LH levels, where MINP dose dependently increased serum LH. This was the only effect which reached statistical significance. There was a tendency towards a positive correlation between MINP and e.g. increasing total testosterone, and the LH:free testosterone ratio. No correlation with cryptorchidism was seen. Also two reviews (Lottrup *et al*, 2006; Swan, 2008) were found discussing this issue. However, in these reviews, no new information related to DINP and effects in humans other than the Main *et al* study was referred to. In an *in vitro* study by Ghisari & Bonefeld-Jorgensen (2009) no effects of DINP on the oestrogen receptor was found.

A recent study by Boberg et al. (2010) also shows results that DINP causes anti-androgenic effects on reproductive development. They found that in male offspring, DINP caused reduced sperm motility and increased prepubertal nipple retention from a dose at 600 mg/kg bw/day, given to pregnant and lactating rats from GD 7 to PND 17. NOAEL can be determined as 300 mg/kg bw/day.

Studies have shown that DINP causes anti-androgenic effects. These effects are though seen at much higher concentrations than for DEHP, DBP, DIBP and BBP and DINP is therefore much less potent compared to these four phthalates and also a preferable alternative.

C.2.2.7 Conclusion on human health DINP

The critical NOAEL for DINP is identified as 15 mg/kg bw/day based on findings of spongiosis hepatis in rats. This NOAEL is not based on endocrine disrupting effects and if dose addition is used the NOAEL for the endocrine disrupting effects should be used to calculate the total exposure and risk.

New information indicates that DINP could be an endocrine distrupting substance, while in other studies no endocrine distruption effects are seen. ECPI questions the validity of some of the studies, including the one by Gray *et al* included in the EU RAR, and they conclude that DINP should not be regarded as an endocrine disrupter.

Studies showing that DINP has anti-androgenic effects on reproductive development, all show that DINP is much less potent than DEHP, DBP, DIBP and BBP and regarding effects on human health DINP would therefore be a better alternative than the four phthalates proposed to be regulated. The NOAEL for antiandrogenic effects could be around 300 mg/kg bw/day. The conclusion from the study by Boberg et al. (2010) is that further safety evaluation of DINP appears warranted, because of the result showing that DINP causes anti-androgenic effects.

C.2.3 Environment risks related to DINP

All is from the EU RAR (2003).

In the aquatic compartment the highest value estimated for a STP outlet is 3.4 mg/l (production site D, worst-case scenario with default values). No PNEC could be derived as no effects at the limit of water solubility could be observed.

No chemical toxic effects of DINP towards fish, invertebrates or algae could be observed in any of the performed long-term tests. No NOECs could be derived. The assessment scheme proposed in EC (1996) can therefore not be used to derive a PNEC for the aquatic compartment. As furthermore, a two-generation study in fish exposed orally was performed, showing no impact on any population parameter, it can tentatively be concluded that DINP does not cause adverse chemical effects towards the aquatic ecosystem.

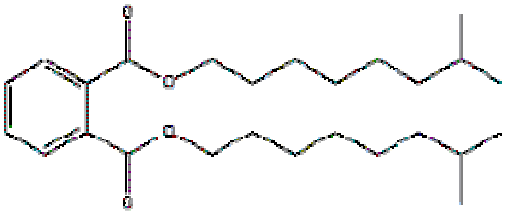
A long-term test has been performed with vertebrates (moorfrog) and a read-across from longterm tests performed with DEHP and DIDP on invertebrates (midge) can be performed. No effects could be observed in any of the test systems. No NOECs could be derived. The equilibrium partitioning model described in the TGD cannot be used to estimate a $PNEC_{sediment}$ as no aquatic PNEC could be derived due to the lack of identified adverse effects. It can therefore tentatively be concluded, that this compound has no adverse effects towards benthic organisms.

In the atmosphere it is so far not possible to realise a biotic assessment in the same way as described for other compartments. No results are available with DINP. No PNEC could be derived from the results available for analogues e.g. DIDP, as no dose response relationship could be established. The absence of adverse effects in the test systems does not give rise for immediate concern though.

C.2.4 Technical and economic feasibility of DINP

The following is all from EU RAR (2003).

Production process
DINP is produced by esterification of phthalic anhydride with isononyl alcohol in a closed system. Isononyl alcohol used in the synthesis of DINP is produced via either the dimerization of butene or the oligomerization of propylene/butene. The reaction rate is accelerated by elevated temperatures (140-250°C) and catalyst. Following virtually complete esterification, excess alcohol is removed under reduced pressure and the product is then typically neutralised, water washed and filtered.



Production, import, export and consumption volumes
Data from producers/importers are included in the IUCLID-database. These are listed:

BASF AG, Germany
Hüls AG, Germany
Exxon Chemical, The Netherlands
ICI C&P, France
Lonza/Alusuisse/Enichem, Italy

Minor producers were identified. ICI C&P in France has stopped production in 1995.

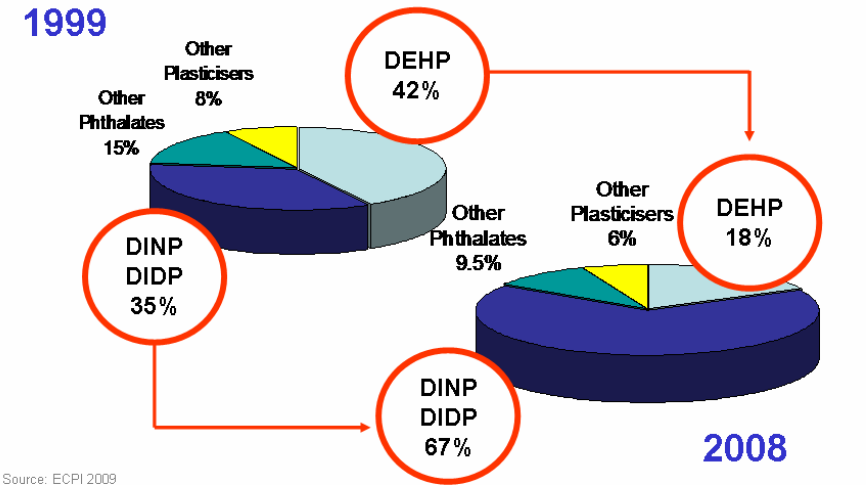
According to the data provided by the producers (ECPI, 1997a), the total production volume in the EU was 185,200 t/a as of 1994. An estimated import volume of 5,400 t/a was obtained from existing inventories from the previous year and approximately 83,400 t/a were exported outside the EU. Consequently, the estimated consumption volume in 1994 is ca. 107,200 t/a. This value is in reasonable agreement with the estimated mean consumption of DINP in Western Europe from 1990-1995 of ca. 121,000 t/a (Legrand, 1996).

Based on estimations by the producers, the evolution of the consumption volumes of DINP (t/a) in Western Europe over the last decades is (Exxon Chemical Europe, 1999):

Year	1964	1970	1975	1980	1985	1990	1994
Volume (t/a)	30,000	40,000	50,000	70,000	80,000	100,000	107,000

A further increase of the consumption of DINP is to be expected over the following years.

In 2009 ECPI reported that the consumption of DINP and DIDP were 35 % of all plasticisers and the DEHP consumption was 42 % of all plasticisers in 1999. In 2008 the total consumption of DINP and DIDP had increased to 67 % and the consumption of DEHP decreased to 18 % of all plasticisers.



Uses

According to ECPI (1997a), approximately 95% of DINP is used in PVC applications. The remaining 5% is used in non-PVC applications. More than half of the DINP used in non-PVC applications involves polymer related uses (e.g. rubbers). The remaining DINP is used in nonpolymer applications including inks and pigments, adhesives, sealants, paints and lacquers and
lubricants.

This is confirmed by the Swedish, Danish and French product registers. In 1994 and 1995, approximately 550-700 tonnes of DINP were included in preparations in the Swedish market (KEMI, 1997). The indicated non-PVC uses are: adhesives and glues (mainly for the industry for transport equipment as well as the industry for wood and wood products), dyestuffs and pigments, paints and varnishes (printing industry and metal coating industry) as well as sealing compounds (industry of transport equipment and construction industry). None of these products were accessible to consumers. Among the 450 tonnes of DINP in preparations in the Danish market in 1996, most are included in adhesives and printing inks (Arbejdstilsynet, 1996).

In the absence of more precise quantitative assignment of use categories, it will be assumed that for non-polymer related uses; the quantities are evenly distributed among adhesives, glues and sealing compounds, inks as well as paints. Taking into account these assumptions, the amounts of DINP used in various applications in Western Europe are listed in Table 41.

Table 41. Estimated amount of DINP used in various PVC and non-PVC applications

Application	**Industry catgory**	**Amount (t/a)**
PVC and uses	Polymer industry (IC=11)	101,500
Non-PVC end uses		5,500
Polymer related	Polymer industry (IC=11)	2,750
Non-polymer related		2,750
Adhesives, glues and sealing compounds	Engineering industry (IC=16)	915
Inks	Pulp, paper and board industry (IC=12)	915
Paints	Paints and varnishes industry (IC=14)	915
Total consumption		107,000

The appropriate use category is softeners (UC=47). The PVC end use split for all phthalates according to Cadogan et al. (1994) as well as estimation for DINP is shown in
Table 42.

Table 42 PVC end use split for all phthalates and estimation for DINP

Application *	Consumption of phthalates [t/a]	Consumption of DINP [t/a]	Percentage
Total consumption in PVC	**877,000**	**101,500**	
Calendering			
Film, sheet and coated products	138,000	15,936	15.7
Flooring, roofing, wall covering	31,000	3,552	3.5
Total		19,488	
Extrusion			
Hose and profile	47,000	5,379	5.3
Wire and cable	251,800	29,020	28.7
Clear, medical, film	62,400	7,125	7.1
Total		41,524	
Injection moulding			
Footwear and miscellaneous	72,800	8,313	8.3
Plastisol spread Coating			
Flooring	92,000	10,658	10.5
General (coated fabric, wall covering, etc.)	100,000	11,571	11.4
Total		22,230	
Other plastisol applications			
Car undercoating and sealants	67,000	7,714	7.6
Slush/rotational moulding etc.	17,000	1,929	1.9
Total		9,643	

Type of PVC products and lifetimes

For the estimation of the releases to the environment through articles containing DINP, it is necessary to estimate the amount of substance included in articles being used outdoors or indoors.

Based on the values reported in
Table 42**,** some products can be recognised for outdoor or indoor use. The flooring is supposed to be used indoors. Car undercoating (7,714 t/a) is used outdoors. Footwear and miscellaneous (8,313 t/a) are assumed to be used outdoors. The use of wires and cables is supposed to be distributed evenly to outdoor (14,510 t/a) and indoor (14,510 t/a) use. For the other types of products, an industry survey for DEHP (di-ethylhexyl phthalate) was performed.

78% of the phthalate containing PVC-products are used in indoor applications and the remaining 22% in outdoor applications (BASF, 1999a). The approximate amounts of DEHP used in PVC for different outdoor applications are found in Table 43, based on figures provided by BASF (1999a).

The respective amounts of DINP can be estimated based on phthalate-market shares of 51% for DEHP and 12% for DINP (Cadogan et al., 1994).
The Danish EPA (Miljöstyrelsen, 1996) reported technical lifetimes for different product groups.

For PVC in cars the lifetime was estimated to be 16 years, for different building materials 10-20 years, and for roof coating 20 years. For roofing material BASF (1999a) gives a lifetime of 20 years. For coil coating 10 years is used (ECPI, 1998b). In this assessment 25 years is used for both roof and wall coating. For cables and wires the lifetime was estimated to be 10-50 years.

In this assessment the average, 30 years, is selected. The technical lifetime for a building is assumed to be 100 years (no reference). No lifetime is available for fabric coating. However, it is assumed to be 10 years.

According to ECPI (1998b), the lifetime for flooring is 10 years. However, according to a producer (Tarkett-Sommer, 1999) is 20 years a more realistic lifetime.

The different lifetimes reported and the values used in this risk assessment are summarised in Table 43.

Table 43 Volumes of DEHP and DINP in different applications of PVC-products and their respective lifetimes.

Application	Tonnage DEHP t/a	Tonnage DINP t/a	Technical lifetime				
			ECPI (1996)	BASF (1999a)	Miljöstyrelsen (1996)	Other	Used in the RAR
Indoor application							
Coated products			7	-	-	-	7
Film & sheet			7	-	1-5 [2]	-	7
Wires & cables		14,510		10-30	30-50	-	30
Hoses & Profiles			10 [1]	-	1-10	20 [3]	20
Floor		10,658	10	-	-	20 [3]	20
Outdoor application							
Roofing material	1,000	230 [4]	-	20	-	-	20
Roofing (coil coating)	5,000	1,150 [4]	-	10	-	-	10
Wires & cables		14,510	-	10-30	30-50	-	30
Coated fabric	21,000	4,850 [4]	-	10	-	-	10
Hoses & Profiles	6,000	1,380 [4]	-	10	-	-	10
Car under-coating		7,714	-	12	16	-	14
Shoe soles		8,313	-	5	-	-	5
Sealings		915					20
Paints & lacquers		915					7

1) Assumed to be the same as for flooring
2) PVC-foils
3) Tarkett-Sommer (1999)
4) Estimated from DEHP, based on market shares

Economic feasibility
The price of DINP is slightly higher per weight than DEHP, about 4 % and since the substitution factor is 1.06 compared to DEHP the effective relative price of DINP is about 11 % higher (source TURI 2006).

C.2.5 Conclusion on DINP

DINP is a phthalate used for many applications and especially after the classification of DEHP the use of DINP has risen.

The health effects of DINP are discussed and there are disagreements of the antiandrogenic effects of DINP. Several studies show that DINP has antiandrogenic effects, and that DINP therefore can be compared to DEHP, DBP, DIBP and BBP. These effects of DINP should be studied more the coming years, but even if DINP should have the same effects as the four phthalates that are proposed to be regulated, the antiandrogenic effects of DINP are seen at much higher concentrations than for the four phthalates that are proposed regulated. DINP would therefore be an easy and relatively cheap alternative to the four phthalates. Even though DINP has shown antiandrogenic activity, these effects are seen at much higher

concentrations compared to the four phthalates, and the antiandrogenic potential should be looked at in more depth.

C.3 Assessment of alkylsulphonic phenylester (ASE)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6

ASE is a mixture of similar esters of sulfonic acids, phenyl and C10 – C18 alkanes, and a liquid at ambient temperatures. It has low solubility in water (2 mg/L) and low volatility (Vp = 0.01 Pa). It is lipophilic with a log K_{OW} >6 (Maag et al., 2010). ASE is not classified according to the CLP Regulation.

C.3.1 Availability of alkylsulphonic phenylester

ASE is available and already in use in several products. The substance has been reported by Danish manufacturers to be used in toy (Maag et al., 2010), and ASE has since 2002 been used in at least one brand of waterbeds (Nilsson et al., 2002). Another study concludes that it is possible to use ASE as a substitute for the normally used phthalate plasticisers in PVC coated textile fabrics such as tents, tarpaulins, rainwear and workwear (Hansen & Lejre, 2002).

C.3.2 Human health risks related to alkylsulphonic phenylester

The following are from Maag et al., 2010.

C.3.2.1 Toxicokinetics ASE

Results from pharmacokinetic studies show that a single oral application by gavage of 1000 mg sulfonic acid, C10-21-alkane, Ph esters/kg bw leads to a concentration of 65 µg sulfonic acid, C10-21-alkane, Ph esters/g fat tissue. After 34 days 4µg sulfonic acid, C10-21-alkane, Ph esters/g was still found in the fat tissue. An elimination half-life of 8 days was calculated for the fat tissue. No accumulation was observed in the liver. 20-30% of the dose was excreted in the faeces within 24 hours. When 100 mg of the substance was administered by gavage, the concentration in fat tissue was 22 µg sulfonic acid, C10-21-alkane, Ph esters/g fat tissue after 3 days and 1 µg/g fat tissue after 14 days. No accumulation was observed in the liver.

C.3.2.2 Acute toxicity ASE

ASE has low acute toxicity by the oral route with LD_{50} reported in the range of 26,380 - 31,650 mg/kg bw in the rat. LD_{50} by the dermal route was found to be > 1,055 mg/kg bw in a rat in a study with no indication of toxicity. ASE was not irritating to rabbit skin when applied to the ears for 24 hours and also not to humans exposed to a saturated patch for 8 hours and followed by a 7 days observation period. Rabbit eyes did not show signs of irritation when exposed to ASE and observed for 7 days.

C.3.2.3 Subchronic toxicity ASE

Subchronic toxicity is studied in a repeated dose 90-day oral toxicity study in the rat. Rats were dosed at 750, 3.000 and 12.000 ppm and a NOAEL was reported at 3,000 ppm corresponding to 228 mg/kg bw in males and 282.6 mg/kg bw in females in spite of significantly dose related absolute and relative liver weights at all dose levels. Effects at the

highest dose level included reduced body weight gain, increased feed (females), increased water consumption (males) and increased kidney weight. No accumulation in the liver was observed in repeated dose toxicity studies of shorter duration (28 to 49 days). Elimination half-life for fat tissue was calculated at 15 days.

C.3.2.4 Mutagenecity ASE

ASE was negative in Ames test, in vitro Mammalian Cytogenetic Test (OECD 473) and in a HGPRT gene mutation assay, all with and without metabolic activation.

C.3.2.5 Toxicity for reproduction

No effects on fertility were seen in what appears to be a three generation reproductive toxicity (fertility) study in rats dosed at 530 mg/kg bw for 6 weeks and observed for 3 months. For the F_0-generation, no effects on fertility are reported. The F_1-generation is reported to have normal weight gain, normal weight of endocrine organs and normal first oestrus. For the F_2 and F_3-generation, no effects on fertility and body weight gain are reported. The study dates back to 1956 and details are not available. It is also not clear if a control group has been included in the study. However, due to the limited information about the study and the high degree of uncertainty, it is not possible to draw any clear conclusions regarding reproductive toxicity from these data.

No other health data was found.

C.3.2.6 Conclusion on human health of ASE
In summary the profile in Table 44 and Table was identified:

Table 44. Summary of data on human health.

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
26,380 - 31,650	> 1,055	ND	No irritation	No irritation	ND

Table 45. Summary of data on human health.

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
228 (m) 282.6 (f)	Negative	ND	-	530	No reliable data	None

The critical NOAEL is 228 mg/kg bw/day, based on reduced body weight gain, increased feed (females), increased water consumption (males) and increased kidney weight. It is not possible to draw any clear conclusions regarding reproductive toxicity from the available data, and the available data did not show any sign of effect on fertility. With these information ASE seem to be a good alternative to the four phthalates.

C.3.3 Environment risks related to alkylsulphonic phenylester

The following are from Maag et al., 2010.

Aerobic biodegradation of ASE is found to be 31% in 28 days. Thus, ASE is not readily biodegradable and its log K_{OW} (>6) is indicative of significant potential for bioaccumulation.

Data on effects of ASE on aquatic organisms are few, however, the data in IUCLID indicate low toxicity to fish in OECD acute test with zebrafish (LC_0 ≥100 mg/L and LC_{50} >10,000 mg/L) and similarly a very low toxicity to crustaceans (*D. magna*); EC >1,000 mg/L and >10,000 mg/L in the two reported tests (OECD acute method). A test with algae (*S. suspicatus*) gave the same result. It is noted that these test and effect concentrations are far above the reported water solubility of ASE (2 mg/L).

No inhibition of the bacteria *Photobacterium phosphoreum* was observed at 500 mg/L in one test while in another <20% inhibition occurred at 1.2 g/L. The EC_{50} for inhibition of activated sludge was >10,000 mg/L.

The main constituents of sulphonic acids, C10-21-alkane, Ph esters are not considered as PBT. They do not meet the P/vP criteria based on screening data but they meet the screening B criteria. Assessment of ecotoxicity (T) was not carried out during this assessment by the PBT Working Group, PBT List No. 82.

No other environmental effect data have been found.

A summary of the environmental effects is shown in Table 46. below.

Table 46. Summary of environmental fate and ecotoxicity data on ASE

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Not readily biodegradable (31% in 28 d)	Log K_{OW} >6	ND (Log K_{OW} indicates low mobility)	LC_{50} (96 h) >100 mg/L	EC_{50} (48 h) >1,000 mg/L	EC_{50} (72 h) >10 mg/l	<20% inhib. at 1.2 mg/l (activated sludge)	ND

ND = No Data

C.3.4 Technical and economic feasibility of alkylsulphonic phenylester

The following are from Maag et al., 2010.

ASE is a mixture of similar esters of sulfonic acids, phenyl and C10 – C18 alkanes (mixture CAS 91082-17-6). It is marketed by Lanxess (formerly Bayer) under the product name Mesamoll.

C.2.4.1 Producer's description (extracts)
The producer Lanxess presents ASE as having the following characteristics (Lanxess, 2009b):

- *Outstanding gelling capacity with a large number of polymers including PVC and polyurethanes, resulting in lower processing temperatures and shorter processing times.*
- *High saponification resistance especially compared to DEHP, due to ASE's chemical structure; this is especially beneficial for articles which come into contact with water and alkalis.*
- *Good compatibility with a large number of polymers such as polyurethane (PU), polyvinyl chloride (PVC), natural rubber (NR), styrene-butadiene rubber (SBR), blends of styrene-butadiene rubber and butadiene rubber (SBR/BR), isobutylene-isoprene rubber (IIR), acrylonitrile-butadiene rubber (NBR) and chloroprene rubber (CR)*
- *Outstanding resistance to weathering and light.*
- *Good dielectric properties which give plasticised PVC outstanding weldability at high frequencies leading to shorter cycle times than with other plasticisers.*

C.3.4.2 Application and market experience

Lanxess has provided information on application areas for ASE among the traditional DEHP, DBP and BBP applications shown in Table 47. The table also indicates the level of market experience in each application area according to Lanxess (2009; interpreted from qualitative text by the report authors). Note that Lanxess has indicated significant market experience for most applications, indicating both general plasticiser characteristics and coverage of several of the special performance characteristics of DBP and BBP.

A Danish study (Nilsson et al., 2002) demonstrated the feasibility of ASE as alternative to phthalates in waterbeds (in the plastisol saturated textile lining), where it is used today.

Table 47. Applications of ASE and level of market experience in each application, data from Lanxess provided for this study.

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	2
Calendering of flooring, roofing, wall covering	4
Extrusion of hose and profile	2
Extrusion of wire and cable	2
Extrusion of miscellaneous products from compounds	2
Injection moulding of footwear and miscellaneous	?
Spread coating of flooring	2
Spread coating of coated fabric, wall covering, coil coating, etc.	2
Car undercoating	2
Non polymer applications:	
Adhesives/sealant, rubber	2
Lacquers and paint	2
Printing ink	2
Production of ceramics	
Substituting for DBP	
Plasticiser in PVC	2
Plasticiser in other polymers	2
Adhesives	2
Printing inks	2
Miscellaneous:	
Sealants	2
PU foam sealants	2
Nitrocellulose paints	2
Film coatings	3
Glass fibre production	
Cosmetics	
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	2
Plastisol coating, for flooring	2
Extrusion or spreadcoating: Leather and cloth coating (e.g. for furniture, shoes, bags, suitcases)	2
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	2
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	2
Coatings and inks (e.g. for car care products, construction, paper, board)	
Adhesives (polymer based, e.g. for construction, paper)	2

1) Market experience categories interpretation: 1) Main alternative on market. 2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

C.3.4.3 Key characteristics

Table 48 below describes some key characteristics of ASE as alternative to DEHP, DBP and BBP.

Table 48. Key characteristics of ASE as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1	NA	
Price (primo 2009)	€1,75/kg	Lanxess (2009)
Price relative to DEHP (≈0.8-1€/kg in 2008/2009; 1€ used for calculations)	175%	
Effective price relative to DEHP	NA	
Compatibility/solubility in PVC		Good (Lanxess, 2009, 2009b)
Permanency (migration, evaporation, extraction)	+	High resistance to extraction by saponification (extraction with soap water), (Lanxess, 2009, 2009b)
Processability (fusing speed and temperature, viscosity, etc.)	+	Low gelling temperature. Faster gellation/fusing speed than DEHP; lower than BBP and DBP (Wilson, 1995; Lanxess, 2009, 2009b)
Limitations in use, if any, noted by supplier		Not suitable for polysulfide based sealants (Lanxess, 2009, 2009b)

Notes: *1: Effectiveness indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. *2: According to Lanxess, 2009, 2009b. NA = not available

Table 49 below shows some performance data for ASE compared to DEHP. As shown, performance parameters are quite close to DEHP's, except for a higher resistance to kerosene (jet-fuel, lipophilic solvent).

Table 49. Technical key parameters of ASE in PVC compared to DEHP (from Sears and Darby, 1982)

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
ASE	72	5.3	0.03	4.8

Wilson (1995) states, that were it not for the higher prices of ASE, it could easily be used as an alternative to the general purpose phthalate plasticisers in a wide range of applications. Its structure gives it some advantages over phthalates for certain processes and aggressive environments. The higher polarity results in faster gelling speed than can be achieved with other plasticisers at similar molecular weight and volatility. ASE has high resistance to degradation from weathering, microorganisms and alkaline media.

C.3.6 Conclusion on ASE

ASE has shown low acute toxicity, negative results in the Ames test and no effects on fertility.

Based on the above mentioned, it seems reasonable to conclude that ASE appears - based on technical observations only - to be an actual general plasticiser alternative to DEHP. The

producer, Lanxess, has indicated significant market experience for most traditional DEHP, DBP and BBP uses, indicating both general plasticiser characteristics and coverage of several of the special performance characteristics of DBP and BBP.

ASE was reported as used for toys by Danish toy manufacturers (with contract production in China), but was not found in the surveys of plasticisers in toys in the Netherlands and Switzerland.

The significantly higher price (75 % more expensive than DEHP) may, however, likely be an impediment to widespread substitution. No information is available on the potential for attaining reduced prices with increased production.

C.4 Assessment of acetyl tributyl citrate (ATBC)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Citrates	Acetyl tributyl citrate	ATBC	77-90-7

ATBC (CAS No. 77-90-7) consists of citrate with three ester bonded butyl groups and one acetyl group bonded to the fourth available oxygen atom. ATBC is a liquid at ambient temperatures and it has a moderate vapour pressure (6.9 Pa at 20 °C). It is sparingly soluble in water (5 mg/L / < 100 mg/L) and quite lipophilic (log K_{OW} = 4.29) (Maag et al., 2010). ATBC is not classified according to the CLP Regulation.

C.4.1 Availability of acetyl tributyl citrate

ATBC is available and already in use in toys and childcare articles. The substance has been reported by Danish manufacturers to be used in toy, and has also been found by analysis in toys and childcare articles (Maag et al., 2010). Furthermore ATCB is also used in medical devices (Karbæk, 2003).

C.4.2 Human health risks related to acetyl tributyl citrate

The following are from Maag et al., 2010.

C.4.2.1 Toxicokinetic ATBC

ATBC is easily absorbed and rapidly metabolised and excreted in the rat. In an absorption study radiolabelled material was recovered at 59 - 70% in urine and cage rinse, 25 - 36% in faeces, 2% expired and 0.36 - 1.26 in tissues and carcass. At least 9 radiolabelled metabolites were found in urine and at least 3 in faeces.

C.4.2.2 Acute toxicity ATBC

ATBC has low acute toxicity by the oral route in rats with LD_{50} reported to exceed 30 g/kg bw. No data on acute toxicity by other routes have been found.

C.4.2.3 Irritation ATBC

ATCB was not irritating to rabbit skin (OECD 404). A study from 1978 in guinea pigs did also not produce irritation, but in an older study from 1955, ATCB produced slight oedema. Patch tests in humans did not produce irritation. ATBC was not irritating to rabbit eyes in a test according to OECD 405. Older studies show slight to moderate irritation. ATCB was not sensitising in guinea pigs or in human volunteers exposed to the substance.

C.4.2.4 Repeated dose toxicity ATBC

Subchronic toxicity has been studied in several repeated dose toxicity studies of varying quality. In a 90 days study (according to OECD 408) in rats exposed to doses of 100, 300 and 1,000 mg/kg/day, NOAEL was reported at 300 mg/kg bw. All rats survived to scheduled necropsy and no treatment related clinical signs were noted throughout the study. Mean body weights were slightly reduced in both sexes in the high dose group and females in the 300 mg/kg dose group beginning at day 28. These findings were however not statistically significant. Increased liver relative weights for both sexes in the 1,000 mg/kg bw dose group and in the males in the 300 mg/kg bw dose group were not associated with any evidence of hepatotoxicity as evaluated by histopathological examination or clinical chemistry. The only other organ weight change was a slightly increased relative kidney weight for males in the high dose group. NOAEL was based on a few statistically significant differences between the control group and animals administered 1,000 mg/kg bw.

Chronic toxicity was studied in a two-year oral feeding study in rats administered 200, 2000 or 20000 ppm in the diet. Transient reduction in body weight gain was observed in all dose groups from week 5 to 15. Because of this unexplained depression in growth rate, two additional groups of 10 rats received ATCB in concentrations of 200 and 2,000 ppm in the diet for one year. As the findings could not be reproduced it was considered to be an artifact. Most other findings were not statistically significant or not considered treatment related. NOAEL was concluded to be 2,000 ppm (100 mg/kg/day) using a conservative approach as the study lacks in detail and is without GLP.

In a 13-week toxicity study with an In Utero Exposure phase, sensitive reproductive and developmental endpoints were examined. Wistar rats received ATBC in the diet in concentration levels of 100, 300 and 1,000 mg/kg/day. F0 males and females were treated for four weeks prior to mating. F1 male and female offspring were exposed in utero and from birth until start of the 13 week study. F1 offspring selected for the study were then treated for 13 weeks. Based on the results a NOAEL for males was established at 100 mg/kg/day and the NOAEL for females at 300 mg/kg/day. At the highest dose level a slight reduction in body weight gain was seen in both sexes, liver weights were increased and hepatic hypertrophy (common finding at high doses of xenobiotics) was seen in males and females. Weak peroxisome proliferase was measured in males at 300 mg/kg/day and in both sexes at 1,000 mg/kg/day. Slight, reversible variations in urinary composition and plasma electrolyte concentration were considered to be due to adaptation to excretion of high levels of test material and/or metabolites and were not considered toxicologically significant.

C.4.2.5 Mutagenicity ATBC

ATBC showed no evidence of mutagenic activity in several Bacterial Reverse Mutation tests (Ames) with and without metabolic activation and also not in Mammalian Cell Gene mutation assays with and without activation. In vitro cytotoxicity was observed in mouse lymphoma cells and less pronounced in HeLa cells. ATCB was not genotoxic in *in vivo/in vitro* unscheduled DNA synthesis study.

C.4.2.6 Toxicity for reproduction of ATBC

ATBC was administered to rats with the diet in a 2-generation reproductive toxicity study in the following doses: 100, 300 and 1,000 mg/kg/day. NOAEL for both parental animals and offspring was found to be 100 mg/kg/day. No treatment-related clinical observations were noted throughout the study in either F0 or F1 parental animals. Body weights the F1 parental males in the 300 and 1,000 mg/kg/day groups were lower that controls and appeared to be related to treatment. Body weights of the F0 females in the 1,000 mg/kg/day group at the end of pregnancy (gestation days 21 or 22) was significantly lower than control values. No reproductive effects were observed in the F0 and F1 generation and no treatment related abnormalities. Slightly lower body weight and slightly higher mortality was observed among

pups in the 300 and 1,000 dose groups, which could be a consequence of reduced water consumption.

No significant treatment related effects on development and embryotoxic effects were observed in a 12 month study in rats.

ATBC showed some signs of neurotoxicity when applied in a 3% acacia to the sciatic nerve in rats and in a 5% suspension of ATBC in 3% gum acacia to the conjunctival sac of the eye of a rabbit. The substance was found to have local anaesthetic action in rabbits and to block neural transmission in rats when placed in contact with a nerve trunk.

C.4.2.7 Conclusion of human health of ATBC

In summary the profile as shown in Table 50 and Table 51 was identified:

Table 50. Summary of data on human health.

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 30,000	> 1,055	ND	No irritation	No / slight irritation	Not sensitising

Table 51. Summary of data on human health.

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
100	Negative	Negative	NOAEL 100	100 (rat)	R and D: No	Decreased bw

R: Reproductive toxicity; D: Developmental toxicity

ATBC was not found to be toxic to reproduction. The critical NOAEL is 100 mg/kg based on reduced body weight gain and increased liver weight. Based on these informations ATBC could be a fine alternative to the four phthalates.

C.4.3 Environment risks related to ATBC (acetyl tributyl citrate)

The following are from Maag et al., 2010.

A number of studies have been conducted to determine the aerobic biodegradability of ATBC. In the modified MITI test with activated sludge inoculum, 80% of the theoretical BOD was reached in 4 weeks. In the static biometer EPA test (EPA 835.3300), ATBC was characterised as readily biodegradable based >60% ThCO2 observed within a 10-14 day window following the lag period. Also in the ASTM D 5338 test, ATBC was found to readily biodegradable as well as ultimately biodedegradable.

A BCF = 250 and a K_{OC} = 1,800 have been calculated for ATBC based on water solubility = 5 mg/L. These values indicate some bioaccumulation potential as well as strong sorption properties i.e. low mobility in soil.

The acute toxicity to fish has been studied using a number of species. The most sensitive end point was found for *Pimephales promelas* larvae (18 hr) in a 7 day static-renewal test (USPEA Method 1000.0). The LC_{50} (48 h) was 2.8 mg/L and the LC_{50} (168 hr) was 1.9 mg/L. In an older (1974) non-guideline flow-through study, the 96 h LC_{50} for *Lepomis*

macrochirus was estimated at 38-60 mg/L and for the mummichog, *Fundalus heteroclitus*, to 59 mg/L (both nominal). ECOSAR modelling using *P. promelas* as model species gave LC_{50} = 1.67 mg/L.

The water flea *Cerodaphnia dubia* was used for testing acute toxicity to daphnia using the USEPA 850.1010 method. The 48 h EC_{50} was determined to be 7.82 mg/L. ECOSAR modelling using *D. magna* as the model species gave an LC_{50} = 0.704 mg/L (48 h). The toxicity to algae has not been tested for ATBC, only estimated by ECOSAR with the green alga *Selenastrum capricornutum*. The 96 hour EC50 was 0.148 mg/L by this method.

A summary of the environmental effects is shown in Table 52 below.

Table 52. Summary of environmental fate and ecotoxicity data on ATBC

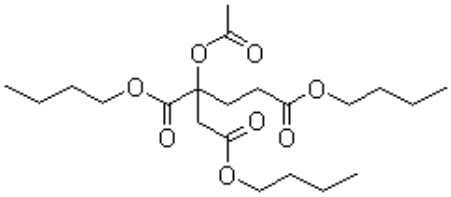
Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumulation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	BCF = 250 (calculated)	K_{OC} = 1,800 (estimated)	LC_{50} (48 h) = 2.8 mg/L LC_{50} (168h) = 1.9 mg/L	EC_{50} (48 h) = 7.82 mg/L	EC_{50} (96 h) = 0.148 mg/L (calculated)	ND	ND

C.4.4 Technical and economic feasibility of acetyl tributyl citrate

The following are from Maag et al., 2010.

ATBC consists of citrate with three ester bonded butyl groups and one acetyl group bonded to the fourth available oxygen atom, see the structure below. The CAS no. is 77-90-7. It is marketed by Vertellus (formerly Morflex), under the product name Citroflex A-4, and by Jungbunzlauer under the products name CITROFOL® BII.

Citrates (ATBC; acetyl tributyl citrate)



C.4.4.1 Producer's description (extracts)

Vertellus (formerly Morflex) has characterised ATBC as follows in their sales material (Vertellus, 2009b): ATBC is compatible with PVC resin, as well as with a range of other polymers. ATBC has mostly been used in products used for sensitive purposes such as medical products food contact products and children's toys. It is, however, too extractable to be useful in some of the applications in the medical area where contact with lipids is important. For such uses, the larger molecule n-butyryltri-n-hexyl Citrate is recommended by Vertellus. The higher molecular weight citric acid esters, including ATBC, are effective replacements for di-(2-ethylhexyl) phthalate (DEHP) and di-(2-ethylhexyl) adipate (DEHA). ATBC also has similar characteristics to some of the lower molecular weight phthalates in a variety of polymers. ATBC is widely used in food contact polymers. It provides many improvements over DBP in cellulose nitrate films, including lower volatility, better resistance to yellowing, and better adhesion to metals. ATBC is effective in solution coating both paperboard and foil. It is a good plasticiser for vinyl toys. ATBC Special is developed and

recommended for medical articles and similar sensitive applications. It is manufactured in a unique, patented process. A special version for use in pharmaceutical coatings is sold as ATBC, PG.

C.4.4.2 Application and market experience

Vertellus has provided information on application areas for ATBC among the traditional DEHP, DBP and BBP applications shown in Table 53. The table also indicates the level of market experience in each application area according to Vertellus (2009).

Table 53. Applications of ATBC and level of market experience in each application (data from Vertellus provided for this study).

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	3
Calendering of flooring, roofing, wall covering	
Extrusion of hose and profile	3
Extrusion of wire and cable	
Extrusion of miscellaneous products from compounds	2
Injection moulding of footwear and miscellaneous	
Spread coating of flooring	
Spread coating of coated fabric, wall covering, coil coating, etc.	
Car undercoating	
Non polymer applications:	
Adhesives/sealant, rubber	2
Lacquers and paint	
Printing ink	2
Production of ceramics	
Other applications (added to list by producer)	
PVC medical articles	2
Toy and childcare articles	1
Substituting for DBP	
Plasticiser in PVC	2
Plasticiser in other polymers	
Adhesives	3
Printing inks	2
Miscellaneous:	
Sealants	3
PU foam sealants	4
Nitrocellulose paints	2
Film coatings	3
Glass fibre production	
Cosmetics	
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	
Plastisol coating, for flooring	
Extrusion or spreadcoating: Leather and cloth coating	
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	
Coatings and inks (e.g. for car care products, construction, paper, board)	3
Adhesives (polymer based, e.g. for construction, paper)	
Nails polish	1

1)Market experience categories interpretation:

1) Main alternative on market.
2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

Note that Vertellus has indicated significant market experience for toys, medical articles, and certain non-polymer applications substituting for DEHP, DBP and BBP, indicating both general plasticiser characteristics and coverage of some of the special performance characteristics of DBP and BBP.

In a study of alternatives to flexible PVC with phthalates, PVC products plasticised with ATBC were reported to match all of the technical requirements (Postle et al., 2000). Similarly, in a lab test of different alternatives to DEHP for their suitability as plasticisers in PVC for medical uses ATBC was found suitable on all technical parameters tested (Karbæk, 2003).

C.4.4.3 Key characteristics

According to ECPI, acetyl tributyl citrate is traditionally used in electrical coatings and casings because of its solvating characteristics. It is also used in inks, hair sprays and aerosol bandages (ECPI, 2009).

The table 54 below (from Vertellus, 2009b) compares various characteristics of medical grade PVC with 50 parts by PVC weight plasticiser (= plasticiser concentration in PVC product 33,3% by weight), 2.5 parts by PVC weight stabiliser (Cl/Zn) and 0.25 parts by PVC weight lubricant (stearic acid), milled and pressure died to sheet (mechanical tests by ASTM methods; Vertellus, 2009b). A-4 is ATBC. T4 and Tf are torsion flex indicators at specific conditions (see Vertellus, 2009b).

Table 54. Comparison of ATBC (=A-4) with DEHP and DEHA for various parameters, from Vertellus (2009).

	DEHP	DEHA	A-4
Hardness	79	78	78
Tensile Strength, psi	2748	1797	2862
Ultimate Elongation, %	395	414	400
100% Modulus, %	1368	1092	1348
T_4 (10,000 Psi), °C	-8.4	-30.8	-7.6
T, (100,000 psi), °C	-38.8	-66.5	-35.6
Brittle Point, °C	-24.5	-56.5	-18.5
Volatile loss (air), %	4.8	7.1	12.1
Volatile loss (A/C), %	3.4	7.6	7.0
Water extraction, %	0.7	1.5	1.2
Soapy water extraction, %	2.7	11.0	9.5
ASTM Oil #3 extraction, %	11.4	34.7	10.9
Silica gel migration, %	12.2	23.0	17.0

As shown, the mechanical tests and oil extraction for DEHP and A-4 = ATBC are very close, while losses to air and aqueous liquids are a factor 2-3 higher for ATBC. According to the

reference, extraction and volatilization properties can be adjusted by adding another plasticiser, for example the less expensive epoxidized soybean oil (ESO).

Table 55 describes some key characteristics of ATBC as alternative to DEHP, DBP and BBP.

Table 55. Key characteristics of ATBC as alternative to DEHP, DBP and BBP.

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1		Equal to or slightly above 1 judged from Shore A hardness.
Price (primo 2009)	NA	
Price relative to DEHP	300%	ExxonMobil (2009); Karbæk (2003)
Effective price relative to DEHP	NA	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Extractability in medical appliances with lipid (fat) contact not found optimal, but losses to oil is found similar to DEHP. Losses to air and aqueous liquids are a factor 2-3 higher than for DEHP (Vertellus, 2009b).
Processability (fusing speed and temperature, etc.)		High solvating
Limitations in use, if any, noted by supplier in data for this study		None noted

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

Table 56 below shows some performance data for ATBC compared to DEHP, DBP and BBP. As shown, ATBC results in a similar hardness as DEHP and BBP, and volatility and extractability higher than DEHP and BBP, but lower than DBP (DBP is the smallest, most mobile molecule of these phthalates).

Table 56. Technical key parameters of ATBC in PVC compared to DEHP, DBP and BBP (from Sears and Darby, 1982).

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
DBP	62	45.4	0.25	9.1
BBP	68	7.7	0.07	3.4
ATBC	73	17.8	0.09	

A Danish toy producing company switched from DINP to ATBC (Citroflex A-4) for all toys for children under 3 years and for toys which are designed to go to the mouth. This particular plasticiser had been given a favourable opinion by the CSTEE for use in toys. However it suffered from a variety of technical drawbacks when compared with DINP. For instance ATBC would not take decoration, it had high migration into adjacent materials leading to swelling and splitting, and there was a need for tooling changes. Development led to the use of a mixture of ATBC, DINCH and DEHT, which could be blended in a variety of combinations to achieve softened PVC that performed to the required standards of safety and reliability with the existing production setup. These blends could be used in many cases as

one-to-one replacements for DINP so that major changes to designs and tooling were not necessary.

Economic feasibility
ATBC price is reported to be approximately 3 times the price of DEHP. Information on the substitution factor is not available.

C.4.5 Other information on ATBC

The Scientific Committee on toxicity, ecotoxicity and the environment (CSTEE) made an opinion on the risk assessment of ATBC used in childrens toy made by the industry. CSTEE concluded toys plasticized with ATBC can be safely mouth by children. As toys mouthed by children must be expected to be an article which gives the highest exposure to the most vulnerable group of consumers, ATBC must be expected to be used safely in other products as well. The opinion does not mention the technical drawbacks with the high migration when used as the only plasticizing substance in toys.

C.4.6 Conclusions on ATBC

ATBC has low acute toxicity, low or slight sensitising, no mutagenic activity and no reproductive effects. Some signs of neurotoxicity were observed.

The performance of ATBC on some parameters seems similar to DEHP, indicating technical suitability for substitution for some purposes. The producer, Vertellus, has indicated significant market experience for toys, medical articles, and certain non-polymer applications substituting for both DEHP, DBP and BBP, indicating both general plasticiser characteristics and coverage of some of the special performance characteristics of DBP and BBP.

ATBC was found in 9% and 10%, respectively, of products analysed in two European studies of large samples of toys and childcare articles. It was also reported as used for toys by Danish toy manufacturers (with contract production in China); used alone it did however not perform adequately in the established toy production setup due to migration to adjacent materials, print resistance, etc. Used in combination with DINCH and DEHT it could be used with no major processing changes.

The higher extractability in aqueous solutions and the higher volatility may reduce the performance of ATBC as a plasticiser in PVC, and could perhaps limit its use for certain applications. Similarly, the extractability in medical appliances with lipid (fat) contact may perhaps limit its use for certain medical applications.

The price of ATBC is significantly higher (200 %) than the price of DEHP, and this may represent a major impediment for its wider use as alternative to DEHP; DBP and BBP.

C.5 Assessment of COMGHA (Glycerides, Castor-oil-mono-, hydrogenated, acetates)

COMGHA consists of fully acetylated monoglycerides of 12-hydroxystearic acid, stearic acid and palmitic acid with a degree of purity of ca. 90.0 - ca. 100.0 % (w/w), usually at 95% (w/w). The main components are 12-acetoxy-octadecanoic acid 2,3-bis(acetoxy)propyl ester (ca. 56%) and 12-acetoxy-octadecanoic acid 2-acetoxy-1-acetoxymethyl-ethyl ester (ca. 28%). A group of minor components consists of fully acetylated monoglycerides of stearic acid and palmitic acid (ca. 10%). The main component of these is octadecanoic acid, 2,3-bis(acetoxy)propyl ester. Furthermore a number of impurities have been identified. The CAS number of the mixture, which is a greasy substance, is 736150-63-3. It has a very low volatility (Vp = 0.00000011 Pa at 25 °C), low water solubility (<0.33 mg/L, later

characterized to be between 60-90 µg/L by Danisco) and is highly lipophilic (log K_{OW} = 6.4). COMGHA is not classified according to CLP.

C.5.1 Availability of COMGHA

COMGHA has not been found used in toys and childcare articles and it is not one of the plasticisers reported by Danish manufacturers to be used (Maag et al., 2010). COMGHA is approved for use in food contact materials and are expected to be used in food contact material, toys and medical devices (GRINDSTED® SOFT-N-SAFE fact sheet).

C.5.2 Human health risks related to COMGHA

The following are from Maag et al., 2010.

C.5.2.1 Toxicokinetics COMGHA

Toxicokinetic studies on COMGHA show that there is no significant absorption of the material across gastrointestinal epithelium. COMGHA appears to be rapidly hydrolysed in the GI tract to acetic acid and fatty acids that undergoes normal fatty acid alpha- and beta-oxidation. COMGHA does not appear to accumulate in tissues. Based on the results from a 90-days oral toxicity study, it was concluded that there were no marked effects on peroxisomal enzyme activities in liver samples at concentration levels of 0.4%, 1.2% and 3.6% in the diet (Maag et al., 2010).

C.5.2.2 Acute toxicity of COMGHA

Acute toxicity (OECD 402) of COMGHA by the dermal route has been studied in rat and LD_{50} found to be > 2,000 mg/kg bw. Other acute toxicity data are not available. COMGHA was not irritating to rabbit skin (OECD 404) and rabbit eyes (OECD 405) and also not a skin sensitizer when studied in a local lymph node assay in mice (OECD 429) (Maag et al., 2010).

C.5.2.3 Repeated dose toxicity of COMGHA

In a 90-day oral toxicity study (OECD 408) with extreme doses: 3, 8.5, 20 ml/kg/day administered by gavage, NOAEL was found to be < 3 ml/kg/day. In a 90-day oral toxicity study (OECD 408) with adequate doses: NOAEL was found to be 5,000 mg/kg/day (Maag et al., 2010).

In a chronic toxicity study (OECD 452) rats were administered doses of 1500, 6000 and 15000 ppm, rising to 25000 and 30000 ppm, in the diet. The concentration of the high dose group was increased during the study to ensure an average achieved dose of at least 1000 mg/kg bw/day. The mean achieved dosage for both genders was 98, 392 and 1333 mg/kg bw/day, respectively. The oral administration of COMGHA to rats for a period of up to 12 months at dietary concentrations of up to 30000 ppm did not result in effects that were considered to represent an adverse effect of treatment. The overall NOAEL for repeated dose oral toxicity (12 months) is 1333 mg/kg bw/day for both genders (information from the registration dossier).

C.5.2.4 Mutagenicity and carcinogenicity of COMGHA

COMGHA was found to be non-mutagenic in the *in vitro* Ames test (OECD 471) and no clastogenic activity was seen in the *In vitro* Mammalian Chromosome Aberration Test

(OECD 473). COMGHA was also non-mutagenic in the *in vitro* Mammalian Cell Gene Mutation Test (OECD 476).

COMGHA was shown to be non-genotoxic when tested in the *in vivo* mammalian erythrocyte micronucleus test (OECD 474). Based on the all the conducted of *in vivo* and *in vitro* genotoxicity studies conducted it was shown that COMGHA does not have genotoxic properties. Further, no evidence of pre-neoplastic changes was seen in an earlier conducted 90-day dietary study in rats (OECD 408) indicating no basis for induction of carcinogenicity (information from the registration dossier).

C. 5.2.5 Toxicity to reproduction

Developmental toxicity was examined in rats and in rabbits (OECD 414) at doses of 100, 300 and 1000 mg/kg bw/day using oral gavage administration of COMGHA. No maternal or developmental toxicity was observed at dose levels up to 1000 mg/kg bw/day and the NOEL for maternal and prenatal developmental toxicity is 1000 mg/kg bw/day (Information from the registration dossier).

Toxicity to reproduction was studied in a two-generation study (OECD 416) in combination with a developmental neurotoxicity study (OECD 426) using rats. A dosing regimen of 0, 1500, 6000 and 15000 ppm in the diet was used. In each generation, animals allocated to the high dose group initially received an intended dietary inclusion levels of 15000 ppm, rising to 20000 ppm and then 25000 ppm during the maturation period and being sustained at the higher inclusion level until termination to ensure an average achieved dose of 1000 mg/kg bw/day.

In the two-generation reproduction/developmental neurotoxicity study, COMGHA was found not to have adverse effects on reproduction and pre- and postnatal development in the rat when administered to two successive generations, including no adverse endocrine disrupting effect using ano-genital distance and nipple count as effect parameters. Furthermore, COMGHA was found not to induce any developmental neurotoxicity in the offspring. The NOEL for adult toxicity and reproduction over the two generations was 25000 ppm, giving exposure of at least 1000 mg/kg bw/day throughout all of the study (lowest average exposure was 1159 mg/kg bw/day in F_0 male). Other than a decrease in spleen weights for female offspring, the NOEL for offspring development was 25000 ppm and this dosage represents a clear NOAEL for offspring development. The NOEL for offspring survival and growth and, also, for developmental neurotoxicity was 25000 ppm.

C.5.2.6 Conclusion on human health COMGHA

In summary of data on human health is given in table 57 and 58 below.

Table 57. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 2,000	ND	ND	No irritation	No irritation	Not sensitising

Table 58. Summary of data on human health.

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
5000	Negative	Negative according to the tests performed	NOEL 1000 mg/kg bw /day	≥1159	No effect	-

5.3 Environmental risks related to COMGHA

COMGHA was found to be readily biodegradable when tested by OECD method 301: 98% degradation occurred in 28 days. The log K_{OW} of 6.4 indicates significant bioaccumulation potential and very low mobility in soil (Maag et al., 2010). COMGHA has very low water solubility, between 60-90 ug/L, indicating that the rate of hydrolysis will be limited by the low water solubility. Therefore, it can be assumed that the rate of hydrolysis in the environment will be very slow. The possible hydrolysis products are glycerol, acetic acid and its constituent acids 12 -acetoxystearic acid and 12 hydroxystearic acid, none of which are suspected to have environmental effects. COMGHA is a strongly adsorbing and binding substance, the adsorption coefficient (Koc) of COMGHA was estimated to be 236178, equal to log Koc=5.4. COMGHA is readily biodegradable and not expected to persist in the environment. The bioaccumulation potential of COMGHA was investigated in zebra fish larvae (*Danio rerio*), from which a conservative estimate of BCF was 981 ± 330, resulting in a log BCF of 2.99 ± 0.14. These data could indicate a potential for bioaccumulation of COMGHA in the environment (information from the registration dossier).

Overall, COMGHA has a high partition coefficient and a high BCF which is characteristic of a bioaccumulative substance. However, COMGHA is a glyceride and therefore inherently metabolizable and bioaccumulation is not expected. Based on the results from the biodegradation study, COMGHA is readily biodegradable and not expected to persist in the environment (information from the registration dossier).

Acute toxicity to zebrafish was tested using OECD 203 but the LC_{50} (96 h) could not be determined as it was higher than the solubility of COMGHA. A no observed effect concentration (NOEC) after 96 h is stated to be 0.28 mg/L () (Maag et al., 2010).

The acute toxicity to daphnia was EC_{50} (48 h) = 0.92 mg/L in the OECD 202 test but COMGHA is, according to the manufacturer, not considered to be acutely toxic at the solubility concentration (<0.33 mg/L, later characterized to be between 60-90 µg/L by Danisco). The 72 hour growth rate inhibition EC_{50} for algae was 26 mg/L.

COMGHA is not considered to be systemically toxic in aquatic species at the solubility concentration. The effect observed in fish and daphnids are considered a physical effect of

the hydrophobic COMGHA rather than manifestations of systemic toxicity. Regarding inhibition of activated sludge respiration (OECD 209), the EC_{20} (and EC_{50}) was >143 mg/L. (Maag et al., 2010).

The potential terrestrial toxicity of COMGHA was tested in a number of studies using earthworm, plants and birds. COMGHA was tested for the acute toxic effects on the earthworm *Eisenia fetida* (OECD 207). Earthworms were exposed for 14 days at a nominal concentration of 1000 mg/kg soil dry weight in a limit test. No mortality was seen and the LC_{50} (14d) was therefore > 1000 mg/kg soil dry weight. In another study, COMGHA was tested for possible effects on terrestrial plants, in which the highest nominal concentration was 1000 mg/kg soil dry weight (OECD 208). The endpoints measured include % emergence, shoot- and root-length for the range-finding test, and % emergence, survival and shoot biomass (dry weight) for the definitive tests. LOECs were 250 mg/kg soil dry weight (nominal) for rape and tomato and 25 mg/kg soil dry weight (nominal) for barley based on decreased plant dry weight. LOECs based on time weighed average concentration weres 41.1 mg/kg soil dry weight for rape and tomato and 6.7 mg/kg soil dry weight for barley. No effects were observed for seed emergence and survival of emerging plants (post emergence). Modelled EC50 estimates based on nominal concentration were approximately 250 mg/kg soil dry weight except for barley for which EC50 was 28.2 mg/kg soil dry weight. Modelled EC50 estimates based on time weighed average concentration was approximately 40 mg/kg soil dry weight except for barley for which EC50 was 6.9 mg/kg soil dry weight. In another study, the acute oral toxicity (LD_{50}) of COMGHA in birds was tested using the Japanese quail. The LD_{50} of COMGHA to the Japanese quail was evaluated to be in excess of 2000 mg/kg bw. The no observed effect level was 2000 mg/kg bw.

Overall, COMGHA did not show toxicity in the terrestrial environment when testing toxicity in earthworm, birds and plants. The observed effect in the terrestrial plant test is evaluated to be due to a physical effect rather than a chemical effect. COMGHA is a strongly adsorbing and binding substance. Therefore, soil-dwelling organisms that feed on soil particles (e. g. earthworms) are most relevant test species. COMGHA is not suspected of having specific effects on arthropods. Therefore, terrestrial toxicity testing to arthropods is assessed not to be relevant (Information from the registration dossier).

COMGHA was tested in a number of long-term aquatic toxicity studies. Due to the very low water solubility of COMGHA, the studies were conducted with radiolabelled COMGHA using concentrations in the range of 60-90 µg/L (70 µg/L was the highest achievable concentration). COMGHA was tested for chronic toxicity including endocrine disrupting effects in zebra fish (OECD 210). The NOEC for embryonic development, hatching, behaviour, growth, survival and change in the sex ratio was ≥ 50.6 µg/L, LOEC was > 50.6 µg/L (highest tested concentration). A NOEC for potential endocrine disrupting effects (vitellogenin (VTG)) was determined to be 32.1µg/L. There was no effect on the sex ratio. In another study, COMGHA was tested for potential endocrine disrupting effects on adult zebra fish (OECD 230). No significant changes in the VTG levels were observed in adult females or males when exposed to COMGHA. LOEC was >46.7 µg/L (highest tested concentration). Based on the fact that COMGHA does not cause any effect on the population relevant endpoint i.e. change in sex ratio and does not cause any significant change in the VTG levels when exposed to adult stages of fish, it is concluded that COMGHA does not cause endocrine disrupting effects in fish. In another study, COMGHA was tested for inhibitory effects on the reproduction of the freshwater crustacean Daphnia *magna* (OECD 211). The NOEC (21 days) was ≥70 µg/L and the EC10 as well as EC50 was be >70 µg/L (highest tested concentration). Overall, COMGHA did not show chronic toxicity or reproductive toxicity, including endocrine disrupting effects, in the solubility range of 60-90 µg/L, using radiolabelled COMGHA (information from the registration dossier).

The bioaccumulation potential of COMGHA was tested in zebra fish larvae (Danio rerio) using a modified in-vitro procedure (OECD 305). Based on the mean bioaccumulation factors, a conservative estimate of the bioaccumulation factor (BCF) was 981 ± 330. These data could indicate a potential for bioaccumulation of COMGHA in the environment. Overall, COMGHA has a high partition coefficient which is characteristic of a bioaccumulative substance. However, COMGHA is a glyceride and therefore inherently metabolizable, thus bioaccumulation is not expected. Based on the results from the biodegradation study, COMGHA is readily biodegradable and not expected to persist in the environment (information from the registration dossier).

A summary of the environmental effects is shown in table 59 and 60 below.

Table 59. Summary of environmental fate and ecotoxicity data on COMGHA

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	log KOW = 6.4	"Immobile in soil"	NOEC(LC10) (96h) = 0.28 mg/L	EC50 (48 h) = 0.92 mg/L	EC50 (72h) = 106 mg/L	EC50 >143 mg/L, activated sludge	LC50>1000 mg/kg soil dry weight

Table 60. Summary of environmental fate and ecotoxicity data on COMGHA

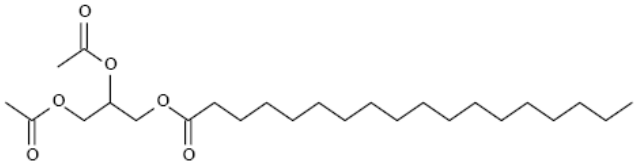
Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	log K_{OW} = 6.4	"Immobile in soil"	NOEC(LC_{10}) (96h) = 0.28 mg/L	EC_{50} (48 h) = 0.92 mg/L	EC_{50} (72h) = 106 mg/L	EC_{50} >143 mg/L, activated sludge	LC50>1000 mg/kg soil dry weight

C.5.4 Technical and economic feasibility of COMGHA

The main constituents are 12-acetoxy-octadecanoic acid 2,3-bis(acetoxy)propyl ester (ca. 56%) designated as “A” and 12-acetoxyoctadecanoic acid 2-acetoxy-1-acetoxymethyl-ethyl ester (ca. 28%) which is the positional isomer of “A”. A group of minor constituents consists of fully acetylated monoglycerides of stearic acid and palmitic acid (ca. 10%). The main component of these is octadecanoic acid 2,3-bis(acetoxy)propyl ester, designated “B”. Furthermore a number of impurities have been identified. The plasticiser consists of castor oil derivatives designated as "COMGHA" (by SCENIHR, 2008) and marketed as GRINDSTED® SOFT-N-SAFE by Danisco. Two of the main substances, “A” and “B” are shown below (from SCENIHR, 2008). In the following technical text, this mixed product is therefore described. The CAS number of the mixture is 736150-63-3.

"A" , 12-acetoxy-octadecanoic acid 2,3-bis(acetoxy)propyl ester ; CAS: 330198-91-9

"B" , octadecanoic acid 2,3-bis(acetoxy)propyl ester ; CAS: 33599-07-4



C.5.4.1 Producer's description (extracts)

Danisco characterises COMGHA/ GRINDSTED® SOFT-N-SAFE as follows (Danisco, 2009b):

COMGHA is an efficient, one-to-one replacement for most conventional plasticisers, such as phthalates. In tests, the quality, durability and functional properties achieved have proven equivalent to phthalate-based solutions. Not only that, COMGHA can be directly applied, without any further alteration to the formulation or processing.

COMGHA has been tested against traditional plasticisers in many PVC applications. Compared with plasticisers such as DEHP, DINP and DOA, the efficiency and reliability of COMGHA is consistently on top.

The main application areas for COMGHA are:

- Food contact applications
- Plastisol production
- Medical devices

COMGHA has been compared with DEHP in numerous flexible PVC applications that perform a sensitive medical role, including tubing and medical film. The test results show that COMGHA meets all requirements in the extrusion, calendaring and injection moulding applications where it has been evaluated. In medical applications where plasticiser migration is a particular concern, COMGHA demonstrated high extraction resistance in aqueous and oily solvents.

Compared with traditional plasticisers such as DEHP and DINP, COMGHA performs consistently well in applications such as vinyl flooring, wallpaper, shrink wrap film, textile dyes, ink applications, adhesives and sealants. In application tests with toys for young children, COMGHA provided the same level of efficiency as DEHP, when measured according to the Shore A scale.

While COMGHA has a significantly higher molecular weight than DEHP and a comparable molecular weight to TOTM, its efficiency remains on top. This makes it a viable candidate for applications that demand low volatility. TGA analysis has shown that COMGHA is considerably less volatile than DEHP under all conditions. Although the novel plasticiser is more volatile than a high end permanency plasticiser as TOTM, the lower loading level required means overall volatility is reduced.

C.5.4.2 Application and market experience

Danisco has provided information on application areas for COMGHA among the traditional DEHP, DBP and BBP applications shown in Table 61. The table also indicates the level of market experience in each application area according to Danisco (2009). Note that COMGHA still has relative moderate market experience, albeit with many examples of full scale usage and pilot/lab scale tests, and significant market experience in plastisol applications and cosmetics.

Table 61. Applications of COMGHA and level of market experience in each application, data from Danisco provided for this study.

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	3
Calendering of flooring, roofing, wall covering	3
Extrusion of hose and profile	3
Extrusion of wire and cable	3
Extrusion of miscellaneous products from compounds	3
Injection moulding of footwear and miscellaneous	3
Spread coating of flooring	2
Spread coating of coated fabric, wall covering, coil coating, etc.	3
Car undercoating	
Non polymer applications:	
Adhesives/sealant, rubber	4
Lacquers and paint	4
Printing ink	3
Production of ceramics	
Substituting for DBP	
Plasticiser in PVC	2
Plasticiser in other polymers	2
Adhesives	4
Printing inks	3
Miscellaneous:	
Sealants	4
PU foam sealants	
Nitrocellulose paints	
Film coatings	
Glass fibre production	4
Cosmetics	2
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	4
Plastisol coating, for flooring	3
Extrusion or spreadcoating: Leather and cloth coating (e.g. for furniture, shoes, bags, suitcases)	2
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	3
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	
Coatings and inks (e.g. for car care products, construction, paper, board)	
Adhesives (polymer based, e.g. for construction, paper)	

*1): Market experience categories interpretation: 1) Main alternative on market. 2) Significant market experience, 3) Examples of full scale experience, 4) Pilot/lab scale experience.

C.5.4.3 Key characteristics

Table 62. below shows selected comparisons from Danisco (2009b) between COMGHA, DEHP and DINP for selected parameters (more parameters are shown in Danisco, 2009b). Note that COMGHA has very similar characteristics as DEHP and DINP.

Table 62. Comparison of COMGHA, DEHP and DINP for selected parameters (Danisco, 2009)

Plasticiser (at 40 phr)	Shore A, after 15 sec	Tensile strength, MPa	100% modulus, MPa	Max. elongation,%
COMGHA	88.0	25.0	9.1	367
DEHP	90.0	22.2	8.5	320
DINP	91.5	24.1	9.3	344

Table 63 describes some key characteristics of COMGHA as alternative to DEHP, DBP and BBP.

Table 63. Key characteristics of COMGHA as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1	≈1	(Danisco, 2009b)
Price	3.5 €/kg	Danisco (2009)
Price relative to DEHP (≈0.8-1€/kg in 2008/2009; 1€ used for calculations)	≈350%	
Effective price relative to DEHP	≈350%	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Much lower extractability than DEHP in acidic water solutions and ethanol/water solutions. Lower extractability in sunflower oil (Danisco, 2009b)
Processability (fusing speed and temperature, viscosity, etc.)		Higher viscosity than DEHP (Danisco, 2009b)
Limitations in use, if any, noted by supplier in data for this study		None noted

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

Both substances in COMGHA are derived from castor oil produced from castor beans. According to Danisco, the beans must be handpicked which sets limits to production volume and cost reductions (Danisco, 2009). Research is therefore ongoing to produce the substances from other, more abundant, biological substrates (Buck Jensen, 2009).

5.5.5 Conclusion on COMGHA

COMGHA has low acute toxicity, no sensitisation, no mutagenic activity and no carcinogenic potential. COMGHA was shown not to have systemic toxic properties after

repeated and chronic oral exposure. Furthermore, COMGHA does not have any reproductive effects, including endocrine disrupting effects, or developmental effects.

COMGHA is highly insoluble in water (60-90 µg/L) and has low potential to cross biological membranes and thus have low potential to accumulate in aquatic species. COMGHA did not show chronic toxicity or reproductive toxicity, including endocrine disrupting effects, in aquatic species and is considered harmless to the environment and to environmental organisms. COMGHA has a high partition coefficient and a high BCF which is characteristic of a bioaccumulative substance. However, COMGHA is a glyceride and therefore inherently metabolizable and bioaccumulation is not expected. Based on the results from the biodegradation study, COMGHA is readily biodegradable and not expected to persist in the environment. COMGHA did not show toxicity in the terrestrial environment when testing toxicity in earthworm, birds and plants.

According to the producer, Danisco, COMGHA still has relative moderate market experience, albeit with many examples of full scale usage and pilot/lab scale tests, and significant market experience in some plastisol application and cosmetics. The producers found technically good performance on key parameters indicating a potential for substituting for DEHP and perhaps for some traditional DBP/BBP uses.

The significantly higher price than DEHP is a major impediment to a wider application of COMGHA. New research initiated needs yet to prove potential for increased production at lower prices.

C.6 Assessment of diethylene glycol dibenzoate (DEGD)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Benzoate	Diethyllene glycol dibenzoate	DEGD	120-55-8

DEGD, also known as Benzoflex 2088, is the esterification product of two benzoate groups with diethylene glycol. It has CAS No. 120-55-8. DEGD becomes liquid at temperatures in the range 24-33 °C and has a very low volatility (Vp = 0,000017 Pa at 25 °C). It has a rather low water solubility of 38.3 mg/L and it is moderately lipophilic with a log K_{OW} of 3.0-3.2. DEGD is not classified according to CLP.

C.6.1 Availability of DEGD

DEGD has not been reported by Danish manufacturers for the use in toys and childcare articles and it has not been identified in analysis of toys and childcare articles. Dibenzoates are reported as alternatives to especially DBP and BBP in certain applications as for example floorings (ECHA, 2009a and ECHA, 2009b).

C.6.2 Human health risks related to DEGD

The following are from Maag et al 2010.

C.6.2.1 Toxicokinetics DEGD

Metabolism of DEGD was studied in Sprague-Dawley CD rats after single oral doses of 50 mg/kg (low level) and 750 mg/kg (high level). Almost all of single oral doses of 50 and 750 mg/kg of DEGD administered to the rats were adsorbed, metabolized and excreted in the

urine within 24 hours of administration. DEGD was metabolized via hydrolysis of the ester bonds to benzoic acid. The free acid was then conjugated with either glycine (major pathway) or glucuronic acid (minor pathway) prior to excretion.

C.6.2.2 Acute toxicity of DEGD

DEGD has low acute toxicity by the oral route in rats with LD_{50} reported at 4,198 mg/kg bw (OECD 401). Dermal LD_{50} in rats was found to be > 2,000 mg/kg bw (OECD 402). An acute inhalation toxicity study in the rat was conducted with DEGD resulting in an LC_{50} > 200 mg/L (4 h).

C.6.2.3 Irritation DEGD

No dermal reaction was reported following a single semi-occlusive application of DEGD to intact rabbit skin for 4 hours. A single instillation of DEGD into the eye of the rabbit elicited transient very slight conjunctival irritation only. No allergic skin reaction was reported in guinea pigs after repeated skin contact (intradermal and topical) using the Magnusson and Kligman method.

C.6.2.4 Repeated dose toxicity DEGD

Subchronic toxicity was studied in a repeated dose 13 week oral toxicity study in the rat (OECD 408). Animals received DEGD in the diet in concentration levels of 250, 1000, 1750 or 2500 mg/kg/day. A NOAEL of 1,000 mg/kg bw was established based on the results of the study. There were no findings of toxicological importance at a dosage of 1,000 mg/kg/day or below. In animals receiving 1,750 or 2,500 mg/kg/day, there was an adverse effect on bodyweight gain, changes in clinical pathology parameters and an increased incidence/degree of haemosiderosis in the spleen. In addition, at 2,500 mg/kg/day, a few treatment-related clinical signs were evident, minimal periportal hepatocyte hypertrophy was noted in both sexes. When selected animals previously receiving 2,500 mg/kg/day were maintained off-dose for 4-weeks, all treatment related changes showed evidence of recovery or recovered completely.

No effects were reported in dogs administered up to 300 mg/kg/day of DEGD in their diet for 90 days.

C.6.2.5 Mutagenicity of DEGD

DEGD did not demonstrate mutagenic potential in bacterial (Ames test, OECD 471/2) or mammalian cell (mouse lymphoma cells, OECD 476) systems with and without metabolic activation. No reponse considered to be indicative of clastogenic activity was observed in a *In-vitro* Mammalian Chromosome Aberration Test in CHL cells (OECD 473).

C.6.2.6 Toxicity for reproduction of DEGD

Prenatal developmental toxicity of DEGD (purity 97.67%) in rats was studied in a test according to US EPA 870.3700 Harmonized Guideline (corresponding to OECD 414). Animals were administered doses of 250, 500 and 1,000 mg/kg/day in the diet. NOEL for maternal toxicity was found to be 1,000 mg/kg/day. At 1,000 mg/kg/day, there were no detectable signs of maternal toxicity; there were no maternal deaths and all females had a live litter at sacrifice. NOAEL for prenatal development was found to be 500 mg/kg/day. A small number of foetuses with cervical ribs was seen at 1,000 mg/kg/day, but not considered indicative of substantial disturbance of morphological development. NOEL for foetal growth and development was 250 mg/kg/day. At 1,000 mg/kg/day mean foetal weights, and consequently litter weight were slightly lower than the control, combined with foetal weight and female foetal weight attaining statistical significance. At 1,000 mg/kg/day 4 foetuses showed cervical ribs, this incidence being higher than the concurrent control and marginally

outside the current background control data. Although the incidence of this finding was relatively low, it is considered that a treatment relationship could not be ruled out.

Reproductive toxicity of DEGD (purity 97.67%) in rats was studied in a 2-generation test according to OECD 416 and at dose levels of 1000, 3300 or 10000 ppm in the diet. corresponding to 50, 165 and 500 mg/kg bw/day based on standard conversion factors. There were no obvious toxicological effects of treatment for the two generations on the general condition of the parental animals although a slight disturbance in the pattern of maternal weight change was noted at 10,000 ppm in both generations and at 3,300 ppm in the F_1 generation. There was no effect on fertility and reproductive performance at any of the dietary inclusion levels in either generation. Litter parameters at birth of the F_1 and F_2 progeny and their survival to weaning showed no apparent detrimental effects of treatment. However, for the F2 offspring at 10,000 ppm there was a reduction in weight gain from birth to weaning. No abnormal findings were apparent at necropsy of the F_O or F_1 parental animals, the post weaned unselected F_1 offspring or the F_2 offspring. Organ weight assessment of the F_O and F_1 parent animals did not suggest any adverse effects on any organs. Assessment of spermatogenesis and histopathology in both parental generations showed that there were no injurious effects on the testes or other reproductive organs. Furthermore, detailed histopathological examination of the tissues from both sexes in both generations did not reveal any adverse effects of treatment. The only possible effect of treatment detected at assessment of organ weights from Fl and F2 offspring was lower absolute and bodyweight relative spleen weights among F2 males and females compared with controls. The evidence from this study suggested that a dietary concentration of 10,000 ppm (500 mg/kg bw/day) should be considered as the NOAEL for the F_O and F_1 parent animals. The NOAEL for the developing offspring is considered to be 3,300 ppm (165 mg/kg bw/day). The NOEL for reproductive parameters is considered to be 10,000 ppm (500 mg/kg bw/day).

Evaluation of estrogenic activity at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days by oral gavage in ovariectomized adult Spraque-Dawley (CD) rats using vaginal cornification and the uterotrophic response as the endpoints demonstrated that DEGD did not exhibit estrogenic activity up to and including the maximally tolerated dose.

C.6.2.7 Summary of human health on DEGD

A summary of data on human health is given in Table 64 and Table 65 below.

Table 64. Summary of data on human health.

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
4,198	> 2,000	> 200mg/L	No irritation	Slight irritation	Not sensitising

Table 65. Summary of data on human health.

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
1,000	Negative	ND	1,000	500 250 (NOEL)	R: No D: Yes	Foetal bw Cervical ribs

The critical NOAEL was 165 mg/kg bw/day showing effects on developing offspring. DEGD has low acute toxicity, low sensibility and no mutagenic activity.

C.6.3 Environmental risks related to DEGD

The following are from Maag et al 2010.

DEGD is found to be readily biodegradable (93% of ThOD in 28 days) in the modified Sturm test (OECD 301B) while in the Closed Bottle Test (OECD 301D) the BOD5/COD ratio was only 0.32 (>0.5 required for ready biodegradability). A K_{OC} of 540 indicates rather low mobility of DEGD in soil, and a moderately high calculated BCF of 120 indicates some bioaccumulation potential.

The aquatic toxicity of DEGD is quite uniform in short term/acute OECD tests between the three main standard groups of test organisms; fish, crustaceans and algae. Thus, the acute (96 h) LC50 to fish (*Pimephales promelas*) is 3.9 mg/L, while the EC50 (48 h) for daphnia is 6.7 mg/L and the 72 hours growth rate-based EC50 for algae (*Seleneastrum capricornutum*, now known as *Pseudokirchneriella subcapitata*) is 11 mg/L. This could indicate a non-specific mode-of-action of DEGD.

The acute toxicity to earthworm (*Eisenia foetida*) (14 days) was found to be >1,000 ppm while the inhibitory effect (IC50) on the bacterium *Pseudomonas putida* could not be determined specifically but only be stated as higher than the highest testable concentration of 10 mg/L. Activated sludge respiration was not inhibited at 100 mg/L.

A summary of environmental fate and ecotoxicity data on DEGD are shown in table 66.

Table 66. Summary of environmental fate and ecotoxicity data on DEGD.

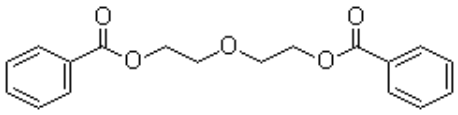
Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	BCF = 120 (calculated)	K_{OC} = 540 (calculated)	LC_{50} (96 h) = 3.9 mg/L	EC_{50} (48 h) = 6.7 mg/L	EC_{50} (72 h) = 11 mg/L	EC_{50} >10 mg/L (P. putida) NOEC ≥100 mg/L, activated sludge	LC_{50} (14 d) >1,000 mg/kg (earthworm)

C.6.4 Technical and economical feasibility of DEGD

The following are from Maag et al 2010.

DEGD is the esterification product of two benzoate groups with diethylene glycol, see structural formula below. Its CAS No. is 120-55-8. It is marketed by Genovique in a mixture with two other dibenzoates under the product name Benzoflex 2088. The two other dibenzoates are dipropylene glycol dibenzoate (DGD; CAS 27138-31-4) and triethylene glycol dibenzoate (CAS 120-56-9). In this section, the mixed product is described. Note that DGD is described separately in the toxicological assessment. DGD is quite similar to DEGD in structure except for two extra methyl groups.

DEGD; diethylene glycol dibenzoate)



(diagram from www.chemblink.com)

Producer's description (extracts)
Benzoflex® 2088 is a high solvating plasticiser primarily known for its exceptional performance in polyvinyl acetate and water-based adhesive systems. It displays good wet tack, set times and open times. It also improves adhesion in acrylic latex caulks (Genovique, 2009b).

In Europe, Benzoflex® 2088 is Genovique's most cost effective replacement for fast fusing phthalate plasticisers used in vinyl applications. It can substitute for phthalates such as BBP, DBP, DIHP and DIBP. It has had its greatest success replacing phthalates in plastisol application, the largest of which is resilient flooring. Over the past five years Benzoflex® 2088 has been established as largest volume non-phthalate, fast fusing plasticiser used in resilient flooring in Europe. Most plastisols were formulated with phthalates in mind, so utilizing an alternative chemistry, like benzoates, requires formulation adjustments (Genovique, 2009).

Application and market experience
Genovique has provided information on application areas for Benzoflex 2088 among the traditional DEHP, DBP and BBP applications shown in Table 67. The table also indicates the level of market experience in each application area according to Genovique (2009; interpreted from qualitative text by the report authors). Note the significant market experience in several of the traditional DBP and BBP specialty plasticiser applications and certain DEHP applications, notably in the non-polymer (adhesives, sealants, etc.) and PVC spread coating (plastisol) application fields.

In a study of plasticiser alternatives for non-PVC applications (COWI, 2000), DEGD was proposed by market actors as a substitute for phthalates in adhesives and sealants.

Table 67. Applications of Benzoflex 2088 (with DEGB) and level of market experience in each application. Data from Genovique provided for this study.

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	4
Calendering of flooring, roofing, wall covering	3
Extrusion of hose and profile	3
Extrusion of wire and cable	3
Extrusion of miscellaneous products from compounds	2
Injection moulding of footwear and miscellaneous	2
Spread coating of flooring	2
Spread coating of coated fabric, wall covering, coil coating, etc.	2
Car undercoating	3
Non polymer applications:	
Adhesives/sealant, rubber	1
Lacquers and paint	2
Printing ink	2
Production of ceramics	
Substituting for DBP	
Plasticiser in PVC	1
Plasticiser in other polymers	
Adhesives	1
Printing inks	
Miscellaneous:	
Sealants	
PU foam sealants	
Nitrocellulose paints	2
Film coatings	
Glass fibre production	
Cosmetics	
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	
Plastisol coating, for flooring	1
Extrusion or spreadcoating: Leather and cloth coating (e.g. for furniture, shoes, bags, suitcases)	2
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	4
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	1
Coatings and inks (e.g. for car care products, construction, paper, board)	
Adhesives (polymer based, e.g. for construction, paper)	1

*1: Market experience categories interpretation: 1) Main alternative on market, 2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

Key characteristics

DEGD is a commonly used benzoate. According to Krauskopf and Godwin (2005), benzoates are generally strong solvates due to the high aromaticity, as are lower molecular weight

phthalates such as BBP. Commercial practice includes the use of up to 10–20% of the plasticiser system as “strong solvating” type plasticisers, such as aryl-alkyl phthalates (e.g. BBP), benzoates, etc.

Table 68. below describes some key characteristics of Benzoflex 2088 as alternative to DEHP, DBP and BBP.

Table 68. Key characteristics of Benzoflex 2088 (with DEGD) as alternative to DEHP, DBP and BBP.

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1		
Price (primo 2009)		
Price relative to DEHP		"Slightly higher" than DEHP and DBP; equivalent to BBP (Genovique, 2009)
Effective price relative to DEHP		
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		App. double volatility as DEHP in plastisols. Three times higher water extraction than DEHP. Much lower organic solvents extraction than DEHP (Genovique, 2009).
Processability (fusing speed and temperature, viscosity, etc.)		Lower gelling/fusing temperature in PVC than with BBP and DEHP. Slightly higher viscosity in plastisols (Genovique, 2009)
Limitations in use, if any, noted by supplier in data for this study		

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

Table 69 below shows some performance data for DEGD (as single substance) compared to DEHP, DBP and BBP. As shown, DEGD has similar characteristics to BBP on these parameters, except for a factor 10 higher extractability in water.

Table 69. Technical key parameters of DEGD in PVC compared to DEHP (from Sears and Darby, 1982).

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
DBP	62	45.4	0.25	9.1
BBP	68	7.7	0.07	3.4
DEGD (as single substance)	69	5.5	0.75	3.4

C.6.5 Conclusions

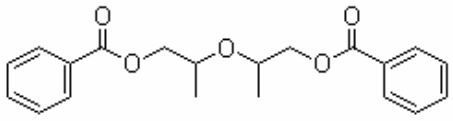
DEGD has low acute toxicity, low sensibility and no mutagenic activity. There were no effect on reproduction and a NOAEL for developing offspring is 165 mg/kg bw/day.

The producer, Genovique, has indicated significant market experience in several of the traditional DBP and BBP specialty plasticiser applications and certain DEHP applications, notably in the non-polymer (adhesives, sealants, etc.) and PVC spread coating (plastisol) application fields. According to the producer, Benzoflex 2088 has become the main non-phthalate alternative to DBP/BBP in vinyl flooring production in Europe. The higher extractability in water may limit its use for some applications. Prices are indicated as "slightly higher" than DEHP and DBP; equivalent to BBP by Genovique.

C.7 Assessment of Dipropylene Glycol Dibenzoate (DGD)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Benzoate	Dipropylene glycol dibenzoate	DGD	27138-31-4

DGD; dipropylene glycol dibenzoate



DGD, CAS No. 27138-31-4, is the esterification product of two benzoate groups with dipropylene glycol. DGD is a liquid and is quite similar to DEGD except for two extra methyl groups. DGD is marketed by Genovique under the product name Benzoflex 9-88, and it is a substance with low volatility (Vp = 0.00016 Pa at 25 °C), a quite low water solubility of 8.9-15 mg/L and relatively lipophilic character with a log K_{OW} of 3.9 (Maag et al., 2010). DGD is not classified according to the CLP Regulation.

C.7.1 Availability of DGD

DGD has not been reported by Danish manufacturers for the use in toys and childcare articles and it has not been identified in analysis of toys and childcare articles. Dibenzoates are reported as alternatives to especially DBP and BBP in certain applications as for example floorings (ECHA, 2009a and ECHA, 2009b).

C.7.2 Human health risk related to DGD

The following are from Maag et al., 2010.

C.7.2.1 Toxicokinetic of DGD

Studies show that DGD is rapidly metabolised and excreted from the body and not accumulated in rats. 70% was excreted in the urine within 48 hours of administration as hippuric acid and about 10% was observed in faeces. Half-life of radiocarbon in the blood was 3 hours and for other organs 2-15 hours.

C.7.2.2 Acute toxicity of DGD

DGD has low acute toxicity by the oral route in rats with LD_{50} reported at 3,914 mg/kg bw (OECD 401). Dermal LD_{50} in rats was found to be > 2,000 mg/kg bw (OECD 402). An acute inhalation toxicity study in the rat was conducted with DGD resulting in an LC_{50} > 200 mg/L (4 h).

C.7.2.3 Irritation DGD

No dermal irritation was reported following a single semi-occlusive application of DGD to intact rabbit skin for 4 hours (OECD 404). A single instillation of DGD into the eye of the rabbit elicited transient very slight conjunctival irritation only (OECD 405). No allergic skin reaction was reported in guinea pigs after repeated skin contact (intradermal and topical) using the Magnusson and Kligman method (OECD 406).

C.7.2.4 Repeated dose toxicity of DGD

Subchronic toxicity was studied in a repeated dose 13 week oral toxicity study in the rat (OECD 408). Animals received DGD in the diet in concentration levels of 250, 1000, 1750 or 2500 mg/kg/day. A NOAEL of 1,000 mg/kg bw (or below) was established based on the results of the study. A few minor intergroup differences were noted at 1,000 mg/kg/day but were insufficient to be of toxicological importance. Higher dosages of 1,750 or 2,500 mg/kg/day were tolerated but the adverse effect on bodyweight was more pronounced, there were increases in circulating enzyme activities, low grade hepatocyte hypertrophy and an increased incidence and degree of hemosiderosis in the spleen in one or both sexes. At 2,500 mg/kg/day, an increased incidence of minimal epithelial hyperplasia was noted in the caecum. When selected animals previously receiving 2,500 mg/kg/day were maintained off dose for 4 weeks, all treatment related effects showed evidence of recovery or recovered completely.

C.7.2.5 Mutagenicity of DGD

DGD did not demonstrate mutagenic potential in bacterial (Ames test, OECD 471/2) or mammalian cell (mouse lymphoma cells, OECD 476) systems with and without metabolic activation. No response considered to be indicative of clastogenic activity was observed in a *In-vitro* Mammalian Chromosome Aberration Test in CHL cells (OECD 473) with and without activation.

C.7.2.6 Toxicity for reproduction of DGD

Prenatal developmental toxicity of DGD (purity 94.84%) in rats was studied in a test according to US EPA 870.3700 (corresponding to OECD 414). Animals were administered doses of 250, 500 and 1,000 mg/kg/day in the diet. NOEL for maternal toxicity was found to be 1,000 mg/kg/day. At 1,000 mg/kg/day, there were no detectable signs of maternal toxicity; there were no maternal deaths and all females had a live litter at sacrifice. NOAEL for prenatal development was found to be 500 mg/kg/day. A small number of foetuses with cervical ribs was seen at 1,000 mg/kg/day. NOEL for foetal growth and development was 250 mg/kg/day. There were no effects of treatment on prenatal survival or growth. At 1,000

mg/kg/day, treatment was associated with a small but definite increase in the number of foetuses with cervical ribs.

Reproductive toxicity of DGD (purity 94.84%) in rats was studied in a 2-generation test according to OECD 416 and at dose levels of 1000, 3300 or 10000 ppm in the diet corresponding to 50, 165 and 500 mg/kg bw/day based on standard conversion factors. There were no obvious toxicological effects of treatment for the two generations on the general condition of the parental animals or on their fertility and reproductive performance.

Litter parameters at birth of the F_1 and F_2 progeny and their survival to weaning showed no apparent detrimental effects of treatment. However, in both the F_1 and F_2 offspring at 10,000 ppm there was a slight reduction in weight gain during days 14-21 of age and this finding may be linked to the transition to direct exposure to the test material as the offspring weaned onto solid diet at the same dietary inclusion levels as their parents.

No abnormal findings were apparent at necropsy of the F_O or F_1 parental animals, the post weaned unselected F_1 offspring or the F_2 offspring. Organ weight assessment of the F_O and F_1 parent animals did not suggest any adverse effects on any organs. Assessment of spermatogenesis and histopathology in both parental generations showed that there were no injurious effects on these testes or other reproductive organs. Furthermore, detailed histopathological examination of the tissues from both sexes in both generations did not reveal any adverse effects of treatment. Regarding survival and growth of the offspring, there were no unequivocal adverse effects. However, a slight reduction in bodyweight gain during days 14 to 21 (F_1 and F_2), likely due to the neonatal consumption of the dam's treated diet, and a slight reduction in spleen weights only observed in the F_2 generation are of questionable toxicological relevance. The evidence from this study suggested that a dietary concentration of 10,000 ppm (500 mg/kg bw/day) should be considered as the NOEL for the F_0 and F_1 parent animals. The NOAEL for survival and growth of the offspring is considered to be 10,000 ppm (500 mg/kg bw/day).

Evaluation of estrogenic activity in a uterotrophic Assay at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days by oral gavage in ovariectomized (ovaries removed and no natural source of oestrogen) adult Spraque-Dawley (CD) rats using vaginal cornification and the uterotrophic response as the endpoints demonstrated that DGD did not exhibit estrogenic activity up to and including the maximally tolerated dose.

C.7.2.7 Summary of human health of DGD

A summary of data on human health is given in Table 64 and Table 71 below.

Table 70. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
3,914	> 2,000	> 200mg/L	No irritation	Slight irritation	Not sensitising

Table 71. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
< 1,000	Negative	ND	1,000 (NOEL)	500 250 (NOEL)	R: No D: Yes	Foetal bw Cervical ribs

D: Developmental

DGD has low acute toxicity, low sensibility and no mutagenic activity. NOAEL for survival and growth of the offspring is 500 mg/kg bw/day in a 2-generation study.

C.7.3 Environmental risks related to DGD

The following are from Maag et al., 2010.

DGD is found to be readily biodegradable (85% of ThOD in 28 days) in the modified Sturm test (OECD 301B) while in the Closed Bottle Test (OECD 301D) the BOD5/COD ratio was only 0.29 (>0.5 required for ready biodegradability). 75% was degraded after 120 days in an anaerobic biodegradation test with a pass level of 60% (US EPA 796.3140, corresponding to OECD 311) and therefore considered to be ultimately biodegradable under anaerobic conditions. The log K_{OW} of 3.9 indicates some bioaccumulation potential and, at the same time, a likely low mobility in soil.

The LC_{50} of fish (*P. promelas*) exposed to DGD for 96 hours was found to be 3.7 mg/L (OECD 203), the 48 hour EC_{50} for daphnia 19.3 mg/L (OECD 202), and the 72 hours growth rate-based EC_{50} for algae (*Seleneastrum capricornutum*, now known as *Pseudokirchneriella subcapitata*) is 11 mg/L. The corresponding 72 h NOEC was 1.0 mg/L. These quite uniform values across three main taxonomic groups could indicate a non-specific mode-of-action of DGD.

The acute toxicity of DGD to earthworm (*Eisenia foetida*) (14 days) was found to be >1,000 ppm while the inhibitory effect (IC_{50}) on the bacterium *Pseudomonas putida* could not be determined specifically but only be stated as higher than the highest testable concentration of 10 mg/L. Activated sludge respiration was not inhibited at 100 mg/L.

A summary of the environmental data is shown in table 72.

Table 72. Summary of environmental fate and ecotoxicity data on DGD

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumulation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready Ultimately biodegradable under anaerobic conditions	Log K_{OW} = 3.9	ND	LC_{50} (96 h) = 3.7 mg/L	EC_{50} (48 h) = 19.3 mg/L	EC_{50} (72 h) = 4.9 mg/L NOEC (72 h) = 1.0 mg/L	EC_{50} >10 mg/L (*P. putida*) NOEC ≥100 mg/L, activated sludge	LC_{50} (14 d) >1,000 mg/kg (earthworm)

C.7.4 Technical and economical feasibility of DGD

Producer's description (extracts)
DGD is a high solvating plasticiser that has been used for many years in a wide variety of applications. Its diverse uses include resilient flooring, adhesives, artificial leather cloth and caulk (Genovique, 2009b).

DGD can be used as a replacement for BBP and DBP in vinyl applications. Its gel fusion temperature is identical to BBP and DBP in vinyl plastisol applications allowing it to be as close to a drop-in replacement for BBP and DBP as possible (Genovique, 2009).

Application and market experience
Genovique has provided information on application areas for DGD among the traditional DEHP, DBP and BBP applications shown in. The table also indicates the level of market experience in each application area according to Genovique (2009; interpreted from qualitative text by the report authors). Note the significant market experience in sealants, adhesives, coatings and inks as well as in PVC spread coating (plastisols), extrusion and injection moulding. DGD seems to be capable of substituting for DEHP, DBP and BBP, indicating coverage of some general plasticiser features as well as some of the special performance characteristics of DBP and BBP.

In a study of plasticiser alternatives for non-PVC applications (COWI, 2000), DGD was proposed by market actors as a substitute for phthalates in adhesives and sealants.

Table 73. Applications of DGD and level of market experience in each application, data from Genovique provided for this study

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	4
Calendering of flooring, roofing, wall covering	3
Extrusion of hose and profile	3
Extrusion of wire and cable	3
Extrusion of miscellaneous products from compounds	2
Injection moulding of footwear and miscellaneous	2
Spread coating of flooring	2
Spread coating of coated fabric, wall covering, coil coating, etc.	2
Car undercoating	3
Non polymer applications:	
Adhesives/sealant, rubber	1
Lacquers and paint	2
Printing ink	2
Production of ceramics	
Substituting for DBP	
Plasticiser in PVC	1
Plasticiser in other polymers	
Adhesives	
Printing inks	
Miscellaneous:	
Sealants	
PU foam sealants	
Nitrocellulose paints	2
Film coatings	
Glass fibre production	
Cosmetics	
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	
Plastisol coating, for flooring	1
Extrusion or spreadcoating: Leather and cloth coating (e.g. for furniture, shoes, bags, suitcases)	
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	4
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	1
Coatings and inks (e.g. for car care products, construction, paper, board)	1
Adhesives (polymer based, e.g. for construction, paper)	

*1: Market experience categories interpretation: 1) Main alternative on market. 2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

Key characteristics

DGD is a commonly used benzoate. According to Krauskopf and Godwin (2005), it's preferred use is in PVC flooring products, owing to its strong solvating strength, and it reportedly controls plasticiser bleeding into asphalt adhesives. In vinyl sheet flooring, the benzoate enhances processing, while the low molecular weight contributes a hardened, stain resistant surface, due to volatilization, similar to the effect of BBP in flooring. Benzoates are generally strong solvents due to the high aromaticity, as are lower molecular weight phthalates such as BBP. Commercial practice includes the use of up to 10–20% of the plasticiser system as "strong solvating" type plasticisers, such as aryl-alkyl phthalates (e.g. BBP), benzoates, etc.

Table 74. describes some key characteristics of DGD as alternative to DEHP, DBP and BBP.

Table 74. Key characteristics of DGD as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1	0.98	TURI (2006, citing industry)
Price	0.73 USD/Lb	TURI (2006, citing industry)
Price relative to DEHP (2006: 0.70 USD/Lb)	2009: "Slightly higher" 2006: 104%	2009: "Slightly higher" according to Genovique (2009); also compared to DBP prices. Equivalent to BBP prices. 2006: Calculated from (TURI, 2006, citing industry); Same price relative to BBP.
Effective price relative to DEHP	102%	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Lover volatility than its competitor BBP (Wilson, 1995).
Processability (fusing speed and temperature, viscosity, etc.)		High solvating, fast fusing; competing with BBP (Wilson, 1995, and others). TURI (2006): Compounding easier than with DEHP; calendering: no issues identified. Some general plasticiser like applications will require blends with slower fusing plasticisers (Genovique, 2009).
Limitations in use, if any, noted by supplier in data for this study		None noted

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

Table 75 below shows some performance data for DGD compared to DEHP, DBP and BBP. As shown, DGD has very similar characteristics to BBP on these parameters, except for a higher extractability in water.

Table 75. Technical key parameters of DGD compared to DEHP, DBP and BBP (from Sears and Darby, 1982)

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
DBP	62	45.4	0.25	9.1
BBP	68	7.7	0.07	3.4
DGD	71	7.9	0.45	2.9

Wilson (1995) states that the consumption of benzoates had so far been minor in Europe although they were well known in the USA, where they were established plasticisers in the PVC flooring industry. Here they were used as fast fusing stain resistant plasticisers. Wilson states the most commercially important benzoate as DGD, which is broadly competitive with BBP, had the advantage of somewhat lower volatility.

Wilson (1995) also emphasises that DGD had replaced much of the previous C4 phthalate use (i.e. DBP, BBP) in PVA adhesives in the USA. In the mid 1990s this substitution had not yet happened in Europe, partly due to higher benzoate prices in Europe, partly due to less regulatory (health and environment) pressure at that time.

Karbæk (2003), who tested DGD and other plasticisers in PVC for medical purposes, found its poor resistance to extraction by water a major drawback for its use in medical applications. Otherwise DGD performed technically well compared to DEHP on all tested parameters, except for tensile strain at break and flexibility at low temperatures.

BBP is mentioned as a critical component in seals for insulating double glaz-ing (BBP Information Centre, 2009), but it has not been specifically investigated whether DGD or other benzoates can substitute for BBP for this particular application.

Prices
DGD has comparable or slightly higher (4 % acc to table 72) prices per weight than DEHP. According to table 72 the substitution factor as a measure for the plasticizing effect pr kg plasticizer is 0.98 compared to DEHP. However DGD is an alternative to BBP, which substitution factor compared to DEHP is 0.94. Thus the the plasticizing effect of DGD is 4 % (1-0.94/0.98) lower than for BBP the effective price is about 8 % higher).

C.7.5 Conclusion on DGD

DGD has low acute toxicity, low sensibility and no mutagenic activity. NOAEL for survival and growth of the offspring is 500 mg/kg bw/day in a 2-generation study.

Genovique, the producer of DGD, has indicated significant market experience in sealants, adhesives, coatings and inks as well as in PVC spread coating (plastisols), extrusion and injection moulding. The qualities of DGD seem especially suitable for substitution of BBP, while it may also substitute for some traditional uses of DEHP and DBP. The fact that DGD has for many years been a well known and much used competitor to BBP in USA, especially in the flooring industry and in PVA adhesives, indicates a clear potential for substituting DGD for BBP, from a technical point of view. DGD and benzoates may already have played a part in the observed reductions (ECHA, 2009c) of BBP usage in Europe.

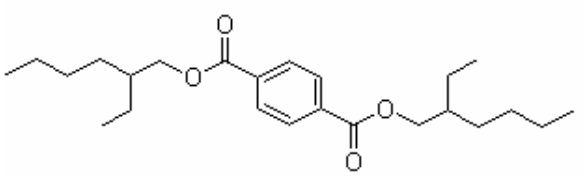
The price of DGD seems also to be largely competitive with low molecular weight phthalates such as BBP. Currently the effective price is about 8% than the price of BBP. As with many

other substitution processes, a price decrease may occur if the market increases in open competition between producers.

C.8 Assessment of di-ethylhexyl-terephthalate (DEHT)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Terephthalate	Di-ethylhexyl-terephthalate	DEHT, DOTP	6422-86-2

Terephthalate, (DEHT; di 2-ethylhexyl terephthalate); CAS no. 6422-86-2



DEHT, CAS No. 6422-86-2, is a phthalate ester stoekiometrically equal to DEHP, i.e. phthalate ester bound to two ethylhexyl groups, but with a different spatial structure, because one of the carboxylic groups is placed differently on the benzyl ring ("tere" means tertiary, or third, because the carboxylic group is placed on the third carbon atom counted from the first carboxyl group).

DEHT is a liquid with low volatility (Vp = 0.0029 Pa) and a very low solubility in water; determined to be 0.4 µg/L in a recent (2002) GLP study using the slow-stir method. Previously reported, higher solubilities (in the low or sub-mg/L range) are now believed to be incorrectly determined. The log K_{OW} of DEHT is as high as 8.39. DEHT is not classified according to the CLP Regulation.

C.8.1 Availability of DEHT

DEHT is already available, and is found in toys and childcare articles (Jansen & Bremmer, 2009). In ECHA, 2009b DEHT is also reported as a suitable alternative for some uses of BBP, in applications where both DEHP and BBP could be used.

C.8.2 Human health assessment related to DEHT

C.8.2.1 Toxicokinetic of DEHT

DEHT has been shown in both *in vitro* and *in vivo* studies to have the potential to undergo complete hydrolysis to yield terephthalic acid and 2-ethylhexanol (2-EH), which are rapidly eliminated. Results of these metabolism studies also indicate DEHT was not well absorbed within the gastrointestinal tract, with 36% of it recovered in the faeces still intact. A study to assess dermal absorption rate indicated that DEHT has a very low potential to penetrate the skin (0.103 µg/cm^2/hr), which further limits systemic exposure potential.

C.8.2.2 Acute toxicity of DEHT

DEHT has low acute toxicity by the oral route in rats with LD_{50} reported at >3,200 mg/kg bw (male rat, no guideline) and >5,000 mg/kg bw (TSCA FHSA Regulations (1979): 16 CFR Part 1500.40). Dermal LD_{50} in male guinea pigs was found to be > 19,670 mg/kg bw. No deaths occurred following inhalation exposure of mice for 4 hr to "saturated" vapours; however, mucosal irritation, loss of coordination and decreased mobility were noted. Recovery occurred in 24 hours.

C.8.2.3 Irritation of DEHT

DEHT was concluded to be a slight dermal irritant in male guinea pigs with no evidence of percutaneous absorption following a single-dose occlusive dermal application and 24 hours exposure. DEHT produces slight irritation to rabbit eyes in a study using a procedure similar to OECD 405.

C.8.2.4 Sensitasation of DEHT

In studies with some limitations, no skin sensitization was observed in humans or guinea pigs.

C.8.2.5 Repeated dose toxicity of DEHT

Subchronic toxicity was evaluated in a 90-days repeated dose toxicity study where rats were fed diets containing DEHT in concentrations of 0.1, 0.5 or 1%. Study was conducted in a manner similar to the one described in the U.S. EPA guideline, 799.9310 TSCA. The only significant treatment related difference between controls and treated animals was increased relative liver weight in the 1.0% dose group. NOEL was 0.5% corresponding to approximately 500 mg/kg/day. In a 21-days repeated oral toxicity study in rats NOEL was also 0.5% (≈ 500 mg/kg/day) based on increased relative liver weight in females at 1.0%.

C.8.2.6 Mutagenicity of DEHT

DEHT did not produce mutagenicity in Ames tests (procedure similar to OECD 471) and also no response considered to be indicative of clastogenic activity in doses up to 1,000 nL/mL in a *In-vitro* Mammalian Chromosome Aberration assays in CHO cells (procedure similar to OECD 473) with and without activation.

C.8.2.7 Carcinogennicity of DEHT

DEHT was evaluated for combined chronic toxicity and carcinogenicity. The test substance was administered in the diets of male and female Fischer-344 inbred rats at concentrations of 20, 142, and 1,000 mg/kg/day. Clinical evaluations revealed no treatment-related signs, however, eye opacities (cataracts) occurred frequently in all groups. At 1,000 mg/kg/day, body weights and female liver weights were reduced. There were no consistent reductions in food consumption. There were no treatment-related effects evident from the gross and histopathologic examinations conducted at 6 and 12 months. At 18 months, two basic lesions of the females in the 1,000 mg/kg/day level appear to be associated with treatment. These were hyperplasia and/or transitional cell adenomas of the urinary bladder and adenomas or adenocarcinomas of the uterus.

In another combined chronic toxicity and carcinogenicity study (industry study) in F-344 rats DEHT was administered in doses of 1,500, 6,000 and 12,000 ppm (79, 324 and 666 mg/kg bw/day (m) and 102, 418 and 901 mg/kg bw/day) in the diet. It was concluded that the oral administration of DEHT via the diet was well tolerated at all dose levels. There was no effect upon tumour incidence and therefore the NOEL for tumorigenicity was at least 666 mg/kg bw/day in males and 901 mg bw/day in females. Toxic responses were confined to low weight gain and food conversion efficiency in males and females receiving 6,000 and 12,000 ppm. Consequently the NOEL for chronic toxicity in the study was 1,500 ppm (79 mg bw/day (m) and 102 mg bw/day (f)).

C.8.2.8 Toxicity for reproduction of DEHT

Reproductive toxicity of DEHT in Sprague-Dawley rats was studied in a 2-generation test according to OECD 416 and at dose levels of 3000, 6000 or 10000 ppm in the diet. The NOAEL for reproductive toxicity was 1.0% in the diet (500-700 mg/kg bw/day for males and 800-1,000 mg/kg bw/day for females; highest dose tested), and the NOAEL for parental and offspring toxicity based on reduced body weight gains was 0.3% (150-200 mg/kg bw/day for

males and 250-300 mg/kg bw/day for females). Mean maternal body weights and body weight gains were reduced for F_0 and F_1 females in the 1.0% group throughout pregnancy and decreased mean terminal body weights were noted in F_1 males and females given 0.6% or 1.0% test material. The results of this study, in conjunction with the 90-day study which also showed no effect of DEHT on histology of reproductive organs indicate that DEHT has a low potential to induce reproductive toxicity.

Developmental toxicity was evaluated in a dietary study following OECD Test Guideline 414 and at dose levels of 3000, 6000 or 10000 ppm in the diet. The NOEL for maternal toxicity was 0.6% (458 mg/kg/day) and the NOEL for developmental toxicity was 1.0% (747 mg/kg/day; highest dose tested).

The ability of DEHT to induce anti-androgenic like effects in male offspring was assessed by giving pregnant rats 750 mg/kg DEHT by gavage on gestation day 14 until postnatal day (PND) 3. No changes indicative of a feminization effect were induced in male pups. NOEL for maternal toxicity and teratogenicity was 750 mg/kg.

Results of an uterotrophic assay in which immature females were given up to 2,000 mg/kg/day DEHT by gavage on PND 19-21 also indicate that DEHT does not possess estrogenic activity.

C.8.2.9 Summary of human health of DEHT

A summary of data on human health is given in 76 and Table 77 below.

Table 76. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 5000	> 19,670	No deaths, saturated vapour (mice)	Slight irritation	Slight irritation	Not sensitising

Table 77. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
500	Negative	Negative	458 (rat)	747 (highest dose tested)	R: Low potential D: No	-

D: Developmental

DETH has low acute toxicity, low sensibility and no mutagenic or carcinogenc activity. The lowest observed NOEL was for maternal toxicity at 458 mg/kg/day.

C.8.3 Environmental assessment of DEHT

In a study from 1986, performed according to the EPA aerobic biodegradation guideline, the biodegradability of DEHT was found to be 56% in 28 days, corresponding to a classification as inherently biodegradable. The BCF of 393 (in oysters, determined in an EPA protocol

study) indicates a medium potential to bioaccumulate, and the K_{OC} of 2,000 a high sorptivity to soil organic matter.

A 7 days flow-through study with *Salmo gairdneri* using acetone to enhance solubility of DEHT resulted in an LC_{50} of ≥ 0.25 mg/L (measured), and a 71 days early life-stage study with the same species gave a NOEC ≥ 0.28 mg/L (measured). The acute toxicity (immobilization) to Daphnia magna was determined in a standard 48 hour static test and found to be EC_{50} ≥ 1.4 mg/L while the 21 days NOEC (reproduction) for D. magna in a flow-through test was 0.76 mg/L (both measured). Inhibition of growth of *Seleneastrum capricornutum* (now known as *Pseudokirchneriella subcapitata*) in the standard 72 hours static test did not occur at the possible test concentrations and the EC_{50} was therefore just stated to be ≥ 0.86 mg/L.

In the OECD 218 test on sediment- dwelling organisms (larvae of *Chironomus riparius*), the EC_{50} after 28 days was determined to ≥ 950 mg/kg while the NOEC for emergence was 180 mg/kg. In a 3 hour activated sludge inhibition test according to OECD 209 the EC_{50} was found to be higher than 10 mg/L.

A summary of the environmental fate is given in table 78.

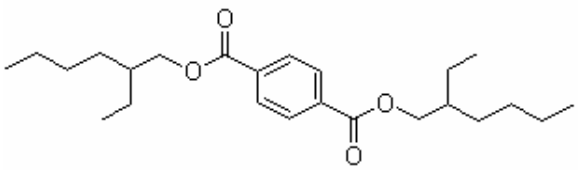
Table 78. Summary of environmental fate and ecotoxicity data on DEHT

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Inherent	BCF = 393	K_{OC} = 2,000	LC_{50} (7 d) > 0.25 mg/L NOEC (71d) ≥ 0.28 mg/L	EC_{50} (48 h) > 1.4 mg/L NOEC (21d) ≥ 0.76 mg/L	EC_{50} (72 h) > 0.86 mg/L	EC_{50} >10 mg/L, activated sludge	ND

C.8.4 Technical and economic feasibility of DEHT

DEHT is a phthalate ester stoekiometrically equal to DEHP, i.e. phthalate ester bound to two ethylhexyl groups, but with a different spatial structure, because one of the carboxylic groups is placed differently on the benzyl ring; see diagram of the structure below ("tere" means tertiary, or third, because the carboxylic group is placed on the third carbon atom counted from the first carboxyl group). DEHT is marketed by Eastman Chemical Company under the product name Eastman 168. It is also marketed by LG Chem under the name LGflex GL300. There are several other producers world wide.

Terephthalate, (DEHT; di 2-ethylhexyl terephthalate); CAS no. 6422-86-2



Producer's description (extracts)
Eastman Chemicals has given the following short presentation of DEHT (Eastman, 2009b): DEHT is a good general purpose plasticiser for PVC, with performance equal or better than

most orthophthalate plasticisers (Eds.: "ortho" is the form for most common phthalate plasticisers, such as in DEHP). It offers good performance properties, good low temperature flexibility, resistance to extraction by soapy water and good non-migration properties. In plastisols, DEHT results in low initial viscosity and good keeping viscosity.

Applications/Uses according to Eastman (2009b):

- Bottle caps and closures
- Coatings
- Coatings for cloth
- Electric connectors
- Flexible film
- Pavement striping compounds
- Sheet vinyl flooring
- Toys
- Traffic cones
- Vinyl compounding
- Vinyl gloves
- Vinyl products
- Vinyl water stops
- Walk-off mats

Application and market experience

Eastman Chemicals has provided information on application areas for DEHT among the traditional DEHP, DBP and BBP applications shown in Table 79. The table also indicates the level of market experience in each application area according to Eastman (2009; interpreted from qualitative text by the report authors). Note that Eastman has indicated significant market experience for all traditional DEHP uses, except car undercoating and production of ceramics. No traditional DBP and BBP applications have been indicated by Eastman, signalling that they consider DEHT as general plasticiser (such as DEHP, DINP and DIDP).

Table 79. Applications of DEHT and level of market experience in each application, data from Eastman Chemicals provided for this study

Application	Market experience *1
Substituting for DEHP	
Polymer applications:	
Calendering of film, sheet and coated products	2
Calendering of flooring, roofing, wall covering	2
Extrusion of hose and profile	2
Extrusion of wire and cable	2
Extrusion of miscellaneous products from compounds	2
Injection moulding of footwear and miscellaneous	2
Spread coating of flooring	2
Spread coating of coated fabric, wall covering, coil coating, etc.	2
Car undercoating	
Non polymer applications:	
Adhesives/sealant, rubber	2
Lacquers and paint	2
Printing ink	2
Production of ceramics	
PVC medical articles	2
Toy and childcare articles	2

*1: Market experience categories interpretation: 1) Main alternative on market. 2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

Key characteristics of DEHT

Table 80 below shows selected comparisons from Eastman (2009c) between DEHT ("168"), DEHP ("DOP"), TXIB and other plasticisers of interest for selected parameters (more parameters are shown in Eastman 2009c). Note that DEHT has similar extraction values to DEHP in oil and hexane, and a factor two lower in soapy water. Volatility at elevated temperatures (expressed as activated carbon extraction) is 50%-67% of the volatility of DEHP. The low temperature flexibility of DEHT in PVC is equal to that of DEHP, whereas 100% modulus is app. 20% higher than for DEHP indicating slightly lower efficiency of DEHT (substitution factor 1.03, cf table 72) than DEHP. DEHT has about half the viscosity of DEHP in plastisols.

Table 80. Comparison between DEHT ("168"), DEHP ("DOP"), TXIB and other plasticisers (from Eastman, 2009c)

Soapy Water Extraction (% wt loss)			Oil Extraction (% wt loss)			Hexane Extraction (% wt loss)		
Eastman Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled
TOTM	0.2	0.1	*TXIB*[f]	14	6.3	*TXIB*[f]	31	24
168	0.5	0.4	DOP	15	8.3	168	31	26
DOP	1.1	0.3	168	16	10	DOP	34	26
TXIB[f]	1.7	1.2	TOTM	24	10	TOTM	35	26
DOA	1.9	0.9	DOA	31	18	DOA	35	29

Activated Carbon Extraction (% wt loss)[c]		
Eastman Plasticizer	Plastisols	Milled
TOTM	0.8	0.5
168	1.2	1.0
DOP	2.5	1.5
DOA	3.7	2.8
TXIB[f]	7.0	6.0

s

Plastisol Viscosity P (Pa·s)[d]		
Eastman Plasticizer	1 Day	21 Days
DOA	45 (4.5)	90 (9.0)
168	75 (7.5)	110 (11.0)
TXIB[f]	95 (9.5)	180 (18.0)
DOP	120 (12.0)	220 (22.0)
TOTM	190 (19.0)	265 (26.5)

Notes: Plastisols contain 60 phr plasticiser; milled and calendered PVC contains 50 phr plasticiser; phr meaning parts per 100 parts hard PVC by weight.
a) Stress at which PVC is elongated 100%. Lower value indicates higher efficiency of plasticiser (ASTM D638). b) Temperature at which the shown stiffness (torsion) is reached (ASTM D1043). c) Indication of volatility at elevated temperatures (ASTM D1203). d) Brookfield viscosity, determined with a number 4 spindle at 6rpm and 23 C.

Table 81 shows another performance data set for DEHT compared to DEHP. As shown, DEHT results in quite similar hardness as DEHP on these parameters. The volatility of DEHT is somewhat lower, and the extractability in water and kerosene is somewhat higher.

Table 81 Technical key parameters of DEHT compared to DEHP (from Sears and Darby, 1982)

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin*1	Shore A hardness*2	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP (PVC2)	73	3.6	0.02	54.7
DEHT (PVC2)	76	1.9	0.09	70.8

Table 82 below describes some generalised key characteristics of DEHT as alternative to DEHP.

Table 82. Key characteristics of DEHT as alternative to DEHP.

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1	1.03	TURI (2006)
Price	0.74 USD/Lb	TURI (2006)
Price relative to DEHP (2006: 0.70 USD/Lb)	106%	TURI (2006) Also according to Krauskopf and Godwin (2005), DEHT is commercially available at similar price as DEHP.
Effective price relative to DEHP	109%	TURI (2006)
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Similar to DEHP, slightly higher permanence on some parameters, slightly lower on others, see above (Eastman, 2009c).
Processability (fusing speed and temperature, viscosity, etc.)	See some values in Table 80 above	Overall very similar to DEHP and DINP (Eastman, 2009; TURI, 2006). Half the viscosity in plastisols as DEHP (advantage of DEHT); slightly higher gelling temperatures than DEHP (Eastman, 2009c).
Limitations in use, if any, noted by supplier in data for this study		Poor weatherability properties for external applications such as roofing and coil coating (Eastman, 2009)

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available.

In practice, terephthalates are more commonly used in the USA than elsewhere (TURI, 2006).

Economic feasibility

The price per kg of DEHT is about 6 % higher than the proce for DEHP. According to table 79 the substitution factor as a measure for the plasticizing effect pr kg plasticizer is 1.03 compared to DEHP. Thus the effective price of DEHT is 9 % (1-1.03*1.06) higher than for DEHP.

C.8.5 Other information on DEHT

Jansen & Bremmer (2009) made a risk assessment on the use of DEHT in toys and childcare articles. The risk assessment was based on the migration of DEHT in artificial saliva, based on a child of 8 kg (10 months) mouthing a toy for 3 hours per day. The risk assessment showed a margin of safety of 7300, and normally a margin of safety of 100 is accepted. This risk assessment indicates that DEHT can be used safely in toys.

C.8.6 Conclusions on DEHT

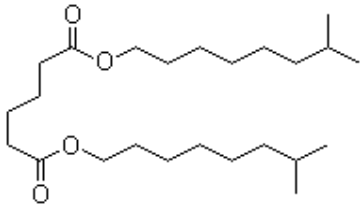
All available information indicates that, technically, DEHT may be a good substitute for most or all traditional DEHP uses. In practice, terephthalates are more commonly used in the USA than elsewhere. DEHT was found in 7% and 10%, respectively, of products analysed in two European surveys of large samples of toys and childcare articles. It was also reported as used for toys by Danish toy manufacturers (with contract production in China).

According to 2006 information, DEHT is about 9% more expensive than DEHP. As with many other substitution processes, a price decrease may occur if the market increases in open competition between producers.

C.9 Assessment of diisononyl adipate (DINA)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Aliphatic dibasic esters	Diisononyl adipate	DINA	33703-08-1

DINA = di isononyl adipate; CAS 33703-08-1



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DINA is formed by an adipate (hexanoic acid) ester bound with two C-9 alkanes. The CAS No. is 33703-08-1. It is a liquid at room temperature and it appears to be moderately volatile (Vp < 10 Pa at 20 °C). It has a low water solubility of < 1 mg/L and is extremely lipophilic with a log K_{OW} = 9.24. DINA is not classified according to the CLP Regulation.

C.9.1 Availability of DINA

DINA has been reported to be used in toys and childcare articles by one Danish manufacturer of toys and childcare articles, and also found in 6 % of samples of toys and childcare articles in a survey from the Netherlands (Maag et al., 2010).

C.9.2 Human health assessment of DINA

The following are from Maag et al., 2010.

C.9.2.1 Toxicokinetics DINA

No data on toxicokinetics have been identified.

C.9.2.2 Acute toxicity of DINA

DINA has low acute toxicity by the oral and dermal route with oral LD_{50} in rats reported at > 5,000 mg/kg bw and dermal LD_{50} in rabbits reported at > 3,160 mg/kg bw.

C.9.2.3 Irratation DINA

DINA was not irritating to rabbit skin (OECD 404) or rabbit eyes (OECD 405). No allergic skin reaction was reported in guinea pigs after repeated skin contact (intradermal and topical) using the Magnusson and Kligman method (OECD 406).

C.9.2.4 Repeated dose toxicity of DINA

Subchronic toxicity was evaluated in a repeated dose 13 week oral toxicity study in the rat at dose levels up to 500 mg/kg/day. NOAEL in males was 500 mg/kg/day. Increased relative kidney weight was observed at 500 mg/kg/day but no change in absolute kidney weight and histopathological changes were seen. It was concluded that there were no significant findings at any dose level. In a 13 week oral toxicity study in dogs fed at doses up to 3% for 8 weeks and 6% for 5 weeks, NOAEL was 1% in the diet corresponding to approximately 274 mg/kg/day. Adverse effects at the high dose included decreased body weight and food

consumption, increased liver weight, elevated enzyme levels, liver and kidney discoloration, and histopathological changes in the liver and kidneys.

C.9.2.5 Mutagenicity of DINA

DINA did not produce mutagenicity in Ames tests or mammalian cell (mouse lymphoma cells) systems with and without metabolic activation. No other data on health effects was found. Bridging data gaps and read across to the structural analogue, adipic acid, bis (2-ethylhexyl) ester (C22) (CAS No. 103-23-1) and three other structurally similar alkyl diesters (C12-C32) was suggested in the US EPA HPV programme for reproductive and developmental toxicity. These diesters show no or low potential for reproductive and developmental toxicity.

C.9.2.6 Summary of human health of DINA

A summary of data on human health is given in Table 6483 and Table 84 below.

Table 83. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 5000	> 3,160	ND	No irritation	No irritation	Not sensitising

Table 84. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
274	Negative	ND	1000 (rat)	-	No data[1)]	-

[1)] Data from structurally similar diesters show low potential for reproductive and developmental toxicity.

A NOAEL of 274 mg/kg bw/day was identified as the lowest NOAEL showing decreased body weight and food consumption, increased liver weight, elevated enzyme levels, liver and kidney discoloration, and histopathological changes in the liver and kidneys at higher dose levels. There are no data on carcinogenicity and reproductive toxicity of DINA.

C.9.3 Environmental assessment of DINA

The following are from Maag et al., 2010.

DINA is readily biodegradable, it has been found to degrade by 82% in 28 days in the modified MITI test (OECD 301C) and by > 90% in the EEC manometric respirometric method. No data on mobility in soil have been found, but the extremely high log K_{OW} of 9.24 indicates a very low mobility. The BCF in a 21 day test with *D. magna* was 1,102-2,031 while in a 35 day test with the blue mussel, *Mytilus edulis*, a BCF = 11,000 was found. No results for BCF in fish have been identified but an estimate from the USEPA gave a BCF = 3.2.

The tests performed with DINA have all been carried out at concentrations exceeding the aqueous solubility i.e. by the use of solubility enhancing solvents such as DMF or acetone. The acute (96 h) toxicity to fish was determined in a test with *Leuciscus idus* according to

DIN 38412. The LC_{50} was > 500 mg/L (nominal), which corresponded to > 2.6 mg/L (measured). The 79/831/EEC static acute immobilization test was conducted with *Daphnia magna*, and an EC_{50} > 100 mg/L was determined. A 21days NOEC > 100 mg/L was found for effects on reproduction of *D. magna* when using the OECD 202, part 2 test method. The 72 hour EC_{50} for algae was > 100 mg/L.

A summary of the environmental fate of DINA is given in table 85.

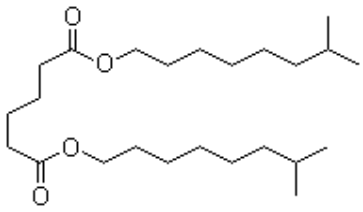
Table 85. Summary of environmental fate and ecotoxicity data on DINA.

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	BCF ≥ 1,100 BCF (estimated) = 3.2	ND	LC_{50} (96 h) > 500 mg/L (nominal) LC_{50} (96 h) > 2.6 mg/L (measured)	EC_{50} (48 h) > 100 mg/L NOEC (21d) > 100 mg/L	EC_{50} (72 h) > 100 mg/L	EC_{50} >10,000 mg/L (*P. putida*) EC_{20} > 1,000 mg/L, activated sludge	ND

C.8.4 Technical and economic feasibility of DINA

DINA is formed by an adipate (hexanoic acid) ester bound with two C-9 alkanes, see diagram. It is marketed by ExxonMobil under the product name Jayflex-DINA, by BASF under the product name Plastomoll DNA, and formerly by Lanxess under the product name Adimoll® DN.

DINA = di isononyl adipate; CAS 33703-08-1



www.chemblink.com

Producers' descriptions (extracts)

BASF has given the following short presentation of DINA (BASF, 2009): DINA is a nearly colourless, clear and practically anhydrous liquid with a hardly noticeable odour. It is soluble in the usual organic solvents and is miscible and compatible with all of the monomeric plasticisers commonly used in PVC. In water DINA is soluble only in very small amounts. Owing to its chemical structure, DINA permits – preferably, in combination with phthalates and polymeric plasticisers – the production of plasticized PVC products with exceptionally good low temperature properties. PVC plasticized with DINA has far less volatility than, for example, PVC plasticized with DOA.

ExxonMobil has given the following short presentation of DINA (ExxonMobil, 2009b):

Improved permanent adipate widely used in a variety of end-uses.

- Low temperature flexibility, low freezing point
- Lower volatility/higher permanence than DOA
- Enhanced viscosity stability and air release properties in plastisols
- Partial replacement for phthalates to improve low temperature flexibility

Applications:

- Films
- Shrink wrap
- Electrical wire jacket

Application and market experience

Neither BASF nor ExxonMobil has wished to provide detailed feedback for this project on DINA's suitability for substituting for DEHP, DBP and BBP.

In a study of plasticiser alternatives for non-PVC applications (Danish EPA, 2001a, 2000), DINA was proposed by market actors as a substitute for phthalates in adhesives, printing inks, paint and lacquer, and rubber.

Key characteristics

The family of adipic acid esters used in PVC applications improves low temperature performance relative to phthalates and gives significantly lower plastisol viscosities in plastisol applications, due to the lower inherent viscosities of the plasticisers themselves (ECPI, 2009).

Table 8 shows some performance data for DINA compared to DEHP, DBP and BBP. As shown, DINA has similar hardness and volatility as DEHP, but higher extractability in water and kerosene.

Table 86. Technical key parameters of DINA compared to DEHP, DBP and BBP (from Sears and Darby, 1982)

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
DBP	62	45.4	0.25	9.1
BBP	68	7.7	0.07	3.4
DINA	72	4.1	0.14	80.4

Table 87 describes some generic characteristics of DINA as alternative to DEHP, DBP and BBP.

Table 87. Key characteristics of DINA as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency(as plasticiser in PVC compared to DEHP)*1	0.98	
Price (primo 2009)	NA	
Price relative to DEHP	150-200% ≈130%	ExxonMobil (2009) Arbeitsgemeinschaft PVC und Umwelt e.V. (2006; statement for adipates in general)
Effective price relative to DEHP	NA	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Far less volatility in PVC than DOA (BASF, 2009); DOA is slightly more volatile than DEHP (Eastman, 2009c)
Processability (fusing speed and temperature, viscosity, etc.)		Enhanced viscosity stability and air release properties in plastisols
Limitations in use, if any, noted by supplier in data for this study		NA

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

C.9.5 Conclusions on DINA

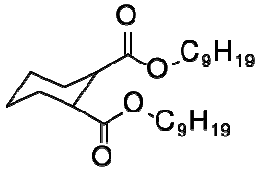
It has not been possible to get detailed application data compared to DEHP, DBP and BBP for DINA from producers. Among the adipates, DEHA (or DOA) is the most used. It was however not included for further investigation in this study because it was reported by SCENIHR (2008) to have reproductive toxicity. Instead DINA was chosen for further study. In PVC DINA has similar hardness and volatility as DEHP, but higher extractability in water and kerosene. DINA has mostly been used for low temperature PVC applications and in PVC film/wrapping. According to the producers, PVC plasticized with DINA has far less volatility than, for example, PVC plasticized with DEHA. DINA seems therefore potentially more suitable than DEHA, from a technical point of view, as an alternative to DEHP than DEHA. DINA seems currently only to have a small European market and data for DINA are scarcer than for DEHA. In fact two producers have ceased marketing of DINA on the European market. However, DINA was found in 6% and 4%, respectively, of products analysed in two European studies of large samples of toys and childcare articles. It was also reported as used for toys by Danish toy manufacturers (with contract production in China). The data available for this study does not allow clear-cut conclusions as regards DINA's suitability as alternative to DEHP, but DINA could perhaps be worth investigating in future technical explorations for alternatives.

The price for DINA is currently 50-100% higher than for DEHP and the substitution factor is close to 1 (table 64) implying that the effective price also is estimated to be 50-100% higher than for DEHP. However, as mentioned the market is small and DINA is used as a specialty plasticiser.

C.10 Assessment of di-isononyl-cyclohexane-1,2-dicarboxylate (DINCH)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Cyclohexanes	Di-isononyl-cyclohexane-1,2-dicarboxylate	DINCH	166412-78-8

DINCH = di-isononyl-cyclohexane-1,2-dicarboxylate; CAS 166412-78-8



DINCH (CAS No. 166412-78-8) is the hydrogenated parallel to DINP, with the difference that the ring structure is cyclohexane (a cyclic alkane) instead of a benzene ring (an aromate). It is a colourless liquid at 20 °C with a very low water solubility of <0.02 mg/L, low volatily (Vp = 0.000022 Pa) and highly lipophilic character (log K_{OW} >6.2). DINCH is not classified according to the CLP Regulation.

C.10.1 Availability of DINCH

DINCH has been reported to be used in toys and childcare articles by Danish manufacturers of toys and childcare articles. DINCH has been reported to be found in 25 % of samples of toys and childcare articles in a survey from the Netherlands and in 48 % in a survey from Austria and Switzerland (Maag et al., 2010).

C.10.2 Human health assessment of DINCH

The following are from Maag et al., 2010.

C.10.2.1 Toxicokinetics of DINCH

DINCH is rapidly absorbed after oral administration and readily eliminated. After 24 hours approximately 80% of the radioactivity is excreted, after 48 hours more than 90% is excreted via urine and mainly via faeces. There is no indication of bioaccumulation. The characterisation of metabolites after oral and intravenous administration of DINCH indicates two main pathways: the partial hydrolysis of DINCH to the mono-isonyl ester followed by conjugation to glucuronic acid, which is the most abundant metabolite in bile, or the hydrolysis of the remaining ester bond to yield free cyclohexane dicarboxylic acid, the predominant urinary metabolite.

C.10.2.2 Acute toxicity of DINCH

DINCH has low acute toxicity by the oral (OECD 423) with LD_{50} in rats reported at > 5,000 mg/kg bw. Dermal LD_{50} in rabbits (OECD 402) was reported at > 2,000 mg/kg bw.

C.10.2.3 Irritation of DINCH

DINCH was slightly irritating to rabbit skin (OECD 404) with mean scores for erythema of 2.0 in one animal and 1.7 in two animals. DINCH was not irritating to rabbit eyes (OECD 405). There was no evidence of skin sensitisation in guinea pigs in a study according to OECD 406.

C.10.2.4 Repeated dose toxicity of DINCH

DINCH was studied in a 28-days oral repeated dose toxicity study (OECD 407) in rats at concentrations of 600, 3000 and 15000 ppm in the diet. Doses of 15,000 ppm caused changes in clinical chemistry parameters in animals of both sexes. Indications of mild renal function impairment (urinary epithelial cells, elevated serum Na+/K+) were observed in male rats. Female rats showed signs that may be associated with hepatic microsomal enzyme induction, characterised by stimulation of γ-glutamyltransferase synthesis and by increased excretion of bilirubin due to stimulation of phase II reactions. NOAEL was established as 3,000 ppm (318 mg/kg bw/day (males) and 342 mg/kg bw/day (females)) in this study, based on the absence of effects on clinical chemistry parameters at this intake level.

In a 90-days oral repeated dose toxicity study (OECD 408) in rats DINCH was administered in the diet at concentrations of 1500, 4500 and 15000 ppm. NOAEL was established at 107.1 mg/kg bw/day (males) and 389.4 mg/kg bw/day (females) in this study, based on kidney weight changes in both sexes and the appearance of degenerated epithelial cells in the urine of males.

In a combined chronic toxicity/carcinogenicity study (OECD 453) rats were administered doses of 40, 200 or 1,000 mg/kg bw/day in the diet. After 24 months of treatment, dose-related follicular cell hyperplasia and increased number of follicular adenomas were observed in the thyroid glands of male rats administered 200 mg/kg bw/day and in both genders administered 1,000 mg/kg bw/day. The thyroid glands are a target organ for the effects of the notified substance in rats. There was a dose-related increased incidence of follicular adenomas in the thyroid gland of mid and high dose male rats and high dose female rats. However, thyroid effects in rats are potentially secondary effects associated with liver enzyme induction and of limited relevance to humans. Such an indirect mechanism is plausible based on the findings of increased GGT activity and lower serum bilirubin levels in this study, and supported by further studies (see special studies below) on enzyme induction and cell proliferation. NOAEL was established at 40 mg/kg bw/day (males) and 200 mg/kg bw/day (females) based on liver weight changes (both sexes) and kidney weight changes (males).

C.10.2.5 Mutagenicity of DINCH

DINCH did not produce mutagenicity in Ames tests (OECD 471) or in *in vitro* mammalian CHO cells with and without metabolic activation. No clastogenic activity was seen in a *c*hromosome aberration assay (OECD 473) with and without activation. DINCH was also not found to be clastogenic or aneuploidogenic in a *in vivo* mouse nucleus test (OECD 474).

C.10.2.6 Toxicity to reproduction of DINCH

Developmental toxicity was examined in rabbits (OECD 414) at doses of 100, 300 or 1,000 mg/kg bw/day in the diet. DINCH did not have any adverse effects on maternal toxicity, gestational parameters or developmental toxicity up to 1,000 mg/kg bw/day. NOAEL was established at 1,000 mg/kg bw/day. In a similar study in rats (OECD 414) with animals dosed at 200, 600 or 1,200 mg/kg bw/day, NOAEL was established at 1,200 mg/kg bw/day due to absence of adverse effects on maternal toxicity and prenatal development. Same conclusions were obtained in a study following elements of OECD 414 and OECD 415 (one-generation reproduction toxicity test) with rats dosed at 750 and 1,000 mg/kg bw/day. NOAEL for maternal and development toxicity was established at 1,000 mg/kg bw/day.

Toxicity to reproduction was studied in a two-generation study with animals dosed at 100, 300 or 1,000 mg/kg bw/day. Under the conditions of this reproduction study, the NOAEL for fertility and reproductive performance was established at 1,000 mg/kg bw/day for F_0 and F_1 generation rats of both genders. The NOAEL for general toxicity was 1,000 mg/kg bw/day (F_0 rats of both genders) and 100 mg/kg bw/day for the F_1 male and female rats (based on

tubular vacuolisation and flaky thyroid follicular colloid). The NOAEL for developmental toxicity (growth and development of offspring) was 1,000 mg/kg bw/day for the F1 and F2 pups.

C.10.2.7 Summary of human health of DINCH

A summary of data on human health is given in Table 64 and Table 89 below.

Table 88. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 5,000	> 2,000	ND	Slight irritation	No irritation	Not sensitising

Table 89. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
107	Negative	Negative	1000 (rat)	1000	R and D: No	-

A NOAEL of 107 mg/kg bw/day was identified as the lowest NOAEL_based on kidney weight changes in both sexes and the appearance of degenerated epithelial cells in the urine of males at higher dose levels. DINCH was not found to be genotoxic, carcinogenic and no effects were observed on reproduction.

C.10.3 Environmental assessment of DINCH

The following are from Maag et al., 2010.

With only 41% degradation in the CO_2 evolution test (OECD 301B), DINCH cannot be classified as readily biodegradable. The BCF of 189 indicates a moderate bioaccumulation potential but 90% depuration of the substance occurred within 1.6 days. The log K_{OW} of >6.2 indicates high sorption potential.

The acute toxicity to fish was tested with zebrafish using the 96 hour static EC-test method and found to be LC_{50} >100 mg/L. Similarly, the acute EC_{50} for daphnia was found to be higher than the highest test concentration in OECD 202 of 100 mg/L. In a 21 days reproduction test (OECD 211) no effects occurred at the highest test level of 0.021 mg/L (measured) and the NOEC was therefore determined to be ≥0.021 mg/L. The rate based 72 hour EC_{50} for algae (*Scenedesmus subspicatus*) was found to be >100 mg/L and the corresponding NOEC ≥100 mg/L.

DINCH was found to be virtually non-toxic to activated sludge (EC_{50} >1,000 mg/L) and to earthworms in the 14 days acute artificial soil test (LC_{50} >1,000 mg/kg).

A summary of the environmental fate is given in table 90.

Table 90. Summary of environmental fate and ecotoxicity data on DINCH

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Not readily biodegradable (41% in 28 d)	BCF = 189	ND	LC_{50} (96 h) >100 mg/L	EC_{50} (48 h) >100 mg/L NOEC (21d) ≥0.021 mg/L	EC_{50} (72 h) >100 mg/L NOEC (72h) ≥100 mg/L	EC_{50} >1,000 mg/L, activated sludge	LC_{50} (14 d) >1,000 mg/kg (earthworm)

C.9.4 Technical and economic feasibility of DINCH

The following are from Maag et al., 2010.

DINCH is the hydrogenated parallel to DINP, with the difference that the ring structure is cyclohexane (a cyclic alkyl hydrocarbon) instead of a benzene ring (an aromate). Its CAS no. is 166412-78-8. It is marketed by BASF as Hexamoll DINCH. There may be other producers outside the EU.

Producer's description (extracts)
BASF describes DINCH as follows (BASF, 2009): The combination of an good toxicological profile and a very low migration rate makes DINCH the plasticiser of choice for medical devices made with soft PVC products such as tubes for internal feeding and haemodialysis bags, respiratory tubes, catheters, gloves and breathing masks.

DINCH is the ideal additive for toys. Thanks to its low migration rate, lack of odour and technical suitability, DINCH is the plasticiser of choice for toys and children's articles such as dolls, inflatables and balls, figurines, modelling clay, swimming aids, baby and childcare articles, wire and cable for toys.

Thanks to its toxicological profile, its low migration rate, and especially the low solubility in water and ethanol, this additive is developed for food contact applications such as cling film, hoses, sealants and cap closures, crown corks, artificial wine corks, gaskets and gloves.

DINCH is also suitable for other applications where safety is needed, also outside PVC: Thermoplastics and polar rubbers, coatings and printing inks, dispersions, adhesives, cosmetics (e.g. nail polish), masterbatches, artificial leather, textile coatings (e.g. rain coats), erasers, film and sheets (e.g. lifestyle bags).

Application and market experience
BASF has not wished to provide detailed feedback for this project on DINCH's suitability for substituting for DEHP, DBP and BBP.

In 2007, BASF raised its DINCH production capacity from 25,000 to 100,000 tonnes/yr (MPW, 2008), indicating an increasing demand.

Key characteristics

Table 91 below describes some key characteristics of DINCH as alternative to DEHP, DBP and BBP.

Table 91. key characteristics of DINCH as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency (as plasticiser in PVC compared to DEHP)*1	NA	
Price	$0.91 /lb	TURI (2006)
Price relative to DEHP (2006: 0.70 USD/Lb)	130%	TURI (2006)
Effective price relative to DEHP	NA	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		Migration levels eight times lower than that of DEHP (MPW, 2008).
Processability (fusing speed and temperature, viscosity, etc.)		NA
Limitations in use, if any, noted by supplier in data for this study		NA

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

C.10.5 Other information on DINCH

Jansen & Bremmer (2009) made a risk assessment on the use of DINCH in toys and childcare articles. The risk assessment was based on the migration of DINCH in artificial saliva, based on a child of 8 kg (10 months) mouthing a toy for 3 hours per day. The risk assessment showed a margin of safety of 5300, and normally a margin of safety of 100 is accepted. This risk assessment indicates that DINCH can be used safely in toys.

C.10.6 Conclusions on DINCH

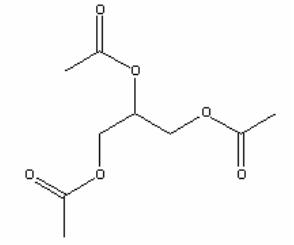
A NOAEL of 107 mg/kg bw/day was identified as the lowest NOAEL based on kidney weight changes in both sexes and the appearance of degenerated epithelial cells in the urine of males at higher dose levels. DINCH was not found to be genotoxic, carcinogenic and no effects were observed on reproduction.
The producer's sales appraisal indicates a relatively wide usage of DINCH for general plasticiser purposes, where safety is prioritised. The production capacity has been raised substantially, indicating a growing demand and market experience. More detailed information of applications substituting for DEHP; DBP and BBP has not been available from the producer. DINCH was the most frequently found plasticiser in two European studies of large samples of toys and childcare articles. It was found in 25% and 48%, respectively, of the analysed products. It was also reported as used for toys by Danish toy manufacturers (with contract production in China). The data available does not allow a closer assessment of DINCH's technical suitability as alternative to DEHP, DBP and BBP.

Sales price in 2006 were 30% than DEHP. Information on the plasticising effect pr kg in relation to DEHP is not available. As with many other substitution processes, a price decrease may occur if the market increases in open competition between producers.

C.11 Assessment on glycerol triacetate (GTA)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Glycerol acetyl esters	Glycerol triacetate	GTA	102-76-1

Glyceryl triacetate (GTA, Triacetin)



GTA ("Triacetin") is an ester of glycerol and three acetate groups. Its CAS no. is 102-76-1. It is a liquid at room temperature with a relatively low vapour pressure of 0.33 Pa at 25 °C. It has high solubility in water (58,000-70,000 mg/L) and a correspondingly low log K_{OW} of 0.21-0.36. GTA is not classified according to the CLP Regulation.

C.11.1 Availability of GTA

The producer of GTA has reported that GTA is suitable as substitute to DBP and BBP in adhesives, inks and coatings, and do not indicate that GTA can be used to any traditional DEHP uses. This is in consistency with the fact that no manufacturers have reported that GTA is used in toys and childcare articles, where the use primarily will be as a plasticiser in PVC (Maag et al., 2010).

C.11.2 Human health assessment of GTA

The following are from Maag et al., 2010.

C.11.2.1 Toxicokinetics of GTA

GTA is rapidly absorbed following ingestion and metabolised like other shorter-chain triglycerides. Several studies confirmed that GTA is hydrolysed to glycerol and acetic acid by digestive enzymes, particularly lipases, liver or plasma carboesterases. GTA is readily hydrolyzed to free glycerol and acetic acid, when incubated with rat intestine in vitro. The chemical infused in dogs undergoes intravascular hydrolysis and the majority of the resulting acetate is oxidized nearly quantitatively. The substance has been shown to be a source of liver glycogen and when fed in amounts equal in caloric value to 15% glucose it was utilised as efficiently as glucose.

C.11.2.2 Acute toxicity of GTA

The acute oral and dermal toxicity of GTA is low. In an oral acute toxicity study in rats (OECD 401), a limit dose of 2,000 mg/kg bw caused no mortality and no signs of systemic toxicity during the 14-day observation period. The LD_{50} in rats by gavage is determined to be >2,000 mg/kg bw for both sexes, and dermal LD_{50} in rabbits and guinea pigs were >2,000 mg/kg bw. Acute inhalation toxicity is considered to be very low, since the LC_{50} in an acute inhalation toxicity study in rats was >1,721 mg/m3 for both sexes (OECD 403) and repeated daily exposure of rats to 73,700 mg/m^3 produced no sign of toxicity after 5 days.

C.11.2.3 Irritation of GTA

GTA was not found irritating to rabbit skin and eyes in studies following OECD 404 and 405. GTA did not induce sensitisation in guinea pigs.

C.11.2.4 Repeated dose toxicity of GTA

In a combined repeat dose and reproductive/developmental screening toxicity test (OECD 422), rats were exposed to 40, 200 or 1,000 mg/kg/day by oral gavage for 44 days from 2 weeks prior to mating for males and for 41 - 48 days from 14 days before mating to day 3 postpartum for females. GTA had no effects on clinical signs, body weight, food consumption, and organ weight or necropsy findings. No histopathological changes ascribable to the compound were observed in either sex. There were no abnormalities in haematological or blood chemical parameters in males. The NOAEL for repeated dose oral toxicity is thus considered to be 1,000 mg/kg bw/day for both sexes.

An inhalation study was conducted in rats given GTA for 90 days at a dose of 249 ppm (2,220 mg/m^3). No signs of toxicity were noted during the exposure. The NOAEL is considered to be 249 ppm (2,220 mg/m^3) for 90 days. Although the inhalation study is considered to be useful, it does not fully comply with the current testing protocol.

C.11.2.5 Mutagenicity of GTA

GTA did not induce gene mutation in Ames test at concentrations up to 5,000 ug /plate (OECD 471 and 472). Induction of chromosome aberrations was observed in the Chinese hamster lung cells at the highest concentration (2.2 mg/mL, 10 mM) in the presence of metabolic activation (OECD 473). Because of high toxicity (75%) that might be caused by low pH (4.9) at the end of the treatment, the chromosomal aberration observed might not be biological relevant. Under un-physiological culture condition, such as low pH, it was reported that the frequency of chromosomal aberrations could be increased. Polyploidy was not induced under any of the conditions tested. Results were equivocal, but taking all data into consideration, GTA could be considered to be non-genotoxic.

C.11.2.6 Repeated dose toxicity and toxicity for reproduction of GTA

The combined repeated dose and reproductive/developmental toxicity study in rats at doses of 40, 200 or 1,000 mg/kg bw/day (OECD 422) showed no statistically significant adverse effects on reproductive parameters (mating index, fertility index, gestation length, numbers of corpora lutea and implantations, implantation index, gestation index, delivery index, parturition and maternal behaviour at delivery and lactation). In addition, there were no significant differences in numbers of offspring or live offspring, the sex ratio, the live birth index, the viability index or body weight. Developmental toxicity, clinical signs of toxicity, and change in necropsy findings were not found in offspring. Therefore, the NOAEL is considered to be 1,000 mg/kg bw/day for parental animals and offspring.

C.11.2.7 Summary of human health of GTA

A summary of data on human health is given in Table 64 and Table 93 below.

Table 92. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 2,000	> 2,000	> 1,721	No irritation	No irritation	Not sensitising

Table 93. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
1000	Negative	ND	1000 (rat)	1000	R and D: No	-

GTA has low acute toxicity and no effects were seen in combined repeated dose and reproductive/developmental toxicity study in rats at doses of 40, 200 or 1,000 mg/kg bw/day**.**

C.11.3 Environmental assessment of GTA

The following are from Maag et al., 2010.

The bulk of data on aerobic biodegradability of GTA suggests that the substance is readily biodegradable, e.g. 77% degradation after 14 days based on BOD, 93% after 28 days based on $ThCO_2$ and 94% after 28 days based on TOC (OECD methods 301B, 301C and 301 D). The K_{OC} of 10.5 indicates high mobility in soil (corresponds well with high water solubility and low log K_{OW}), and the calculated BCF = 1.3 implies an insignificant bioaccumulation potential.

The acute toxicity of GTA to fish has been studied on a number of species such as *Pimephales promelas, Oryzias latipes, Cyprinus carpio, Brachydanio rerio and Leuciscus idus*. In the test with *O. letipes*, the lethal level was not reached at the highest test concentration of 100 mg/L, and among the other species the LC_{50} ranged from 165 to 300 mg/L with *P. promelas* being the most sensitive species (tested with OECD 203, DIN38412 or ISO 7346/2 (conforming to OECD 203)). In a prolonged test with *O. latipes* the 14 days LC_{50} was > 100 mg/L (nomimal) (OECD 204).

EC_{50}'ies for acute toxicity to *D. magna* range from 380 to 811 mg/L with the most sensitive result being obtained with the DIN 39412 Teil 11 test, which conforms to OECD 202. The 21 days NOEC on reproduction of *D. magna* was 100 mg/L in the OECD 211 test. Inhibition of growth of *Seleneastrum capricornutum* (now known as *Pseudokirchneriella subcapitata*) in the standard 72 hours static test (OECD 201) could not be determined but was > 1,000 mg/L. The NOEC (72 h) was determined to be 556 mg/L. The toxicity to bacteria, *Pseudemonas putida*, was determined using the EN ISO 10712 guideline. A 16 hours NOEC = 3,000 mg/L was determined.

A summary of the environmental fate is given in table 94.

Table 94. Summary of environmental fate and ecotoxicity data on GTA

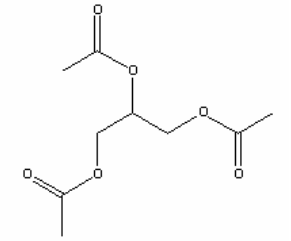
Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumulation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Ready	BCF = 1.3	K_{OC} = 10.5	LC_{50} (96 h) = 165 mg/L LC_{50} (14 d) > 100 mg/L	EC_{50} (48 h) = 380 mg/L NOEC (21d) = 100 mg/L	EC_{50} (72 h) > 940 mg/L NOEC (72h) = 556 mg/L	NOEC (16h) = 3,000 mg/L (*P. putida*)	ND

C.11.4 Technical and economic feasibility of GTA

The following are from Maag et al., 2010.

GTA is an ester of glycerol and three acetate groups, see diagram below. Its CAS no. is 102-76-1. It is marketed by Lanxess and Eastman under the name Triacetin.

Glyceryl triacetate (GTA, Triacetin)



Producer's description (extracts)
Lanxess (2009) presents GTA as follows: Triacetin is used for the solidification of acetyl cellulose fibres in the manufacture of cigarette filters. The water content must be kept constant to achieve constant solidification. Triacetin is also used as a support for flavourings and essences in the food industry and as a plasticiser for chewing gum. In technical applications, Triacetin is used for example as a core sand binder in the metal foundry sector. Another application is inks and printing inks. Triacetin is used as a highly effective plasticiser for cellulose-based plastics.

The major features of Triacetin are:

- good suitability for the solidification of acetyl cellulose fibres for the manufacture of cigarette filters
- very good dissolving power for a number of organic substances
- good plasticising effect for various plastics such as cellulose acetates or celluloseacetobutyrates
- good plasticising effect for cellulose-based paints
- good compatibility with natural and synthetic rubber
- good light resistance

Eastman presents GTA as follows (Eastman, 2009d): GTA is used as a plasticiser for cellulosic resins and is compatible in all proportions with cellulose acetate, nitrocellulose, and ethyl cellulose. GTA is useful for imparting plasticity and flow to laminating resins, particularly at low temperatures, and is also used as a plasticiser for vinylidene polymers and copolymers. It serves as an ingredient in inks for printing on plastics, and as a plasticiser in nail polish. GTA is approved by the FDA for food packaging and many other food-contact applications.

Application and market experience
Lanxess has provided information on application areas for GTA among the traditional DEHP, DBP and BBP applications shown in Table 95. The table also indicates the level of market experience in each application area according to Lanxess (2009; interpreted from qualitative text by the report authors). Note that Lanxess has indicated significant market experience for adhesives, coatings and inks and examples of full scale experience in a number of other non-polymer traditional DBP and BBP uses. Lanxess has not indicated use of GTA in any traditional DEHP uses.

Table 95. Applications of GTA and level of market experience in each application, data from Lanxess provided for this study

Application	Market experience *1
Substituting for DEHP	
Plasticiser in PVC	
Plasticiser in other polymers	
Adhesives	2
Printing inks	3
Miscellaneous:	
Sealants	
PU foam sealants	
Nitrocellulose paints	3
Film coatings	
Glass fibre production	
Cosmetics	
Substituting for BBP	
Polymer applications:	
General PVC (e.g. for moulded plastic parts)	
Plastisol coating, for flooring	
Extrusion or spreadcoating: Leather and cloth coating (e.g. for furniture, shoes, bags, suitcases)	
Films, calendering (e.g. for packaging, calendered flooring, wall covering, etc.)	
Non polymer applications:	
Sealants (polysulfide based, polyurethane foam sealants, acrylic based; e.g. for windows, construction etc.)	
Coatings and inks (e.g. for car care products, construction, paper, board)	2
Adhesives (polymer based, e.g. for construction, paper)	

*1: Market experience categories interpretation: 1) Main alternative on market.
2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience.

Key characteristics

Table 96 describes some key characteristics of GTA as alternative to DEHP, DBP and BBP.

Table 96. Key characteristics of GTA as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency (as plasticiser in PVC compared to DEHP)*1	NA	
Price (primo 2009)	€1,50/KG	Lanxess (2009)
Price relative to DEHP (≈0.8-1€/kg in 2008/2009; 1€ used for calculations)	150%	
Effective price relative to DEHP	NA	
Limitations in use, if any, noted by supplier in data for this study		NA

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, while plasticisers needing lower concentration (more effective) have lower values, and plasticisers needing higher concentrations have higher effectiveness value. NA = not available

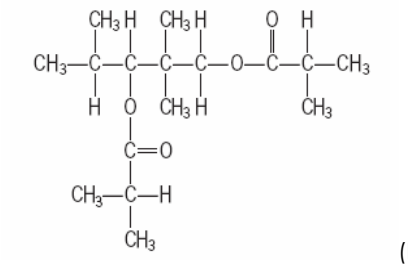
C.11.5 Conclusions on GTA

According to a producer, GTA can substitute for DBP and BBP in adhesives, inks and coatings. The price of GTA is about 50%, higher, (source Lanxess 2009) that DEHP (and DBP and BBP). The data available does not allow a closer assessment of GTA's technical suitability as alternative to DEHP, DBP and BBP.

C.12 Assessment of trimethyl pentanyl diisobutyrate (TXIB)

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Other alkyl esters	Trimethyl pentanyl diisobutyrate	TXIB	6846-50-0

TXIB



(Eastman, 2009e)

TXIB is an ester of the branched alkane trimethyl pentanyl with two butyrate groups. Its CAS no. is 6846-50-0. The vapour pressure is only 0.089 Pa, the water solubility low (1-2 mg/L; 15 mg/L) and the lipophilicity quite high (log K_{OW} is 4.1 or more). TXIB is not classified according to the CLP Regulation.

C.12.1 Availability of TXIB

TXIB has not been reported to be used in toys and childcare articles by Danish manufacturers of toys and childcare articles. But TXIB has been reported to be found in 14 % of samples of toys and childcare articles in a survey from the Netherlands and in 11 % in a survey from Austria and Switzerland (Maag et al., 2010).

C.12.2 Human health assessment of TXIB

The following are from Maag et al., 2010.

C.12.2.1 Toxicokinetics of TXIB

TXIB was rapidly adsorped, metabolized and excreted. The major route of elimination was urine (47 – 72% total dose) within 5 - 10 days and the majority of this occurring in the first 72 hours. Radioactivity in faeces accounted for 14 – 31% of the dose with elimination being essentially complete by 7 days with the majority isolated after 48 hours. Radiolabeled CO_2 was not detected. In total, excretions accounted for 95-99% of the dose. Residual radioactivity of treated animals approached control by two weeks. Identification of metabolites showed the faeces to contain both 2,2,4-trimethyl pentanediol (TMPD) and TXIB-3-14C indicating esterase cleavage of the two isobutyrates. A small potion of the absorbed material in the urine was unchanged TXIB-3-14C while the majority consisted of metabolites consistent with complete cleavage to the glycol (TMPD) parent molecule. Although much of the urinary metabolite was unidentified it does, nonetheless, represent rapidly cleared material.

C.12.2.2 Acute toxicity of TXIB

TXIB has low acute toxicity by the oral route with LD_{50} in rats reported at > 3,200 mg/kg bw. Dermal LD_{50} in rabbit (OECD 402) was reported at > 2,000 mg/kg bw. LC_{50} in rats exposed to 0.12 mg/L or 5.3 mg/L for 6 hours was > 5.3 mg/L.

C.12.2.3 Irritation of TXIB

Skin irritation was studied in guinea pigs and rabbits. In guinea pigs TXIB was slightly irritating to the skin and in rabbits (OECD 404) no irritation was observed. TXIB was not found irritating to rabbit eyes (OECD 405).

C.12.2.4 Sensitasation of TXIB

TXIB was not found sensitising to skin in guinea pigs in a test following a protocol similar to OECD 406. In a study on human volunteers using a modified Draize procedure , TXIB was found non-irritating ad did not induce any evidence of sensitisation.

C.12.2.5 Repeated dose toxicity of TXIB

TXIB was studied in a 103 days oral repeated dose toxicity study in rats receiving concentrations of 1% and 1.0% in the diet. There was a slight, significant increase in the relative liver weights in the 1.0% group and in the absolute liver weights in the 1.0% male group when compared to controls. NOAEL was established at 0.1%. In another study rats were exposed to the same concentrations for 52 or 99 days (I: 52 days TXIB diet, II: 99 days TXIB diet, III: 52 days TXIB + 47 days control diet or 52 days control diet + 47 days TXIB diet). From the study it appeared that high doses of TXIB cause significant adaptive changes in the rat liver, and these changes are reversible if the animal is returned to normal diet. NOAEL was 0.1%. In dogs (beagles) fed a diet with 0.1, 0.35 or 1.0% TXIB for 13 weeks, NOAEL was established at 1.0% because of no toxicological significant findings related to treatment.

C.12.2.6 Mutagenicity of TXIB

TXIB did not produce mutagenicity in Ames tests (Japanese guideline) or in *in vitro* mammalian CHL cells (Japanese guideline) with and without metabolic activation.

C.12.2.7 Toxicity for reproduction of TXIB

In a combined repeat dose and reproductive/developmental screening toxicity test (OECD 422), rats were exposed to 30, 150 or 750 mg/kg/day. A NOEL at 30 mg/kg/day was

established based on effects on liver and kidneys in the higher dose groups. When evaluating the reproductive toxicity, NOEL (Parental) and NOEL (F_1 offspring) was established at 750 mg/kg/day, as no effects on reproduction (mating, fertility and oestrus cycle, dams during pregnancy and lactation, pubs after birth) related to treatment were observed.

In another combined study (OECD 421 with additional sperm motility asssessment) TXIB was administered in doses of 91, 276 or 905 mg/kg /day in males and 120, 359 and 1,135 mg/kg/day in females. Statistically significant reproductive effects observed in the high dose group include reduced number of implantation sites, reduced mean litter weights on postnatal (PND) 0, reduced mean number of live pubs on PND 4, decreased mean absolute epididymal sperm counts, and reduced absolute and relative testicular sperm counts. The mean number of live pubs per litter was also reduced on PND 0. NOAEL for reproductive and developmental toxicity was 276 mg/kg/day on males and 359 mg/kg/day in females based on reduced number of implantation sites and reduced mean number of live pubs on PND 0.

C.12.2.8 Summary of human health of TXIB

A summary of data on human health is given in Table 64 and Table 98 below.

Table 97. Summary of data on human health

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
> 3,200	> 2,000	> 5,3 mg/L (6h)	No / slight irritation	No irritation	Not sensitising

Table 98. Summary of data on human health

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
1000	Negative	ND	1000 (rat)	276 (m)	R: Yes D: No	Red. no. implantations sites and mean no. of live pubs

TXIB has low acute toxicity and is a slight irritant. The lowest NOAEL observed is 276 mg/kg bw/day based on reduced number of implantation sites and reduced mean number of live pubs on PND 0. TXIB is potential reproductive.

C.12.3 Environmental assessment of TXIB

The following are from Maag et al., 2010.

TXIB was found to be inherently biodegradable in the OECD 301C test and the BCF (carp) determined to be in the range 5.2-31 (OECD 305C), i.e. a low bioaccumulation potential. The BCF of 4.1 (or more) indicates a low mobility of TXIB in soil.

Acute toxicity of TXIB to fish: LC_{50} (96 h) = 18 mg/L (OECD 203; *Oryzias latipes*). In one test (OECD 202) on *Daphnia magna* the EC_{50} was found to be 300 mg/L while in another EC_{50} >1.46 mg/L. The 14 days NOEC (reproduction) for *D. magna* was determined to 3.2 mg/L in a test basically performed according to OECD principles but apparently not fulfilling all test requirements. The 72 hours biomass-based EC_{50} for algae (*Seleneastrum capricornutum*, now known as *Pseudokirchneriella subcapitata*) has been determined to 8.0 mg/L using the OECD 201 method. The corresponding 72 h NOEC was 5.3 mg/L.

A summary of the environmental fate is given in table 99.

Table 99. Summary of environmental fate and ecotoxicity data on TXIB

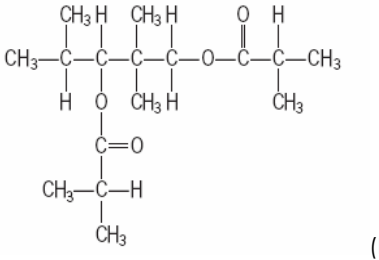
Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestrial
Inherent	BCF = 5.2-31	ND	LC_{50} (96 h) = 18 mg/L	EC_{50} (48 h) >1.46 mg/L NOEC (14d) = 3.2 mg/L	EC_{50} (72h) = 8.0 mg/L NOEC = 5.3 mg/L	ND	ND

C.12.4 Technical and economic feasibility of TXIB

The following are from Maag et al., 2010.

TXIB is an ester of the branched alkane trimethyl pentanyl with two butyrate groups. Its CAS no. is 6846-50-0. Until 2006, it was marketed by Eastman as TXIB plasticiser, but since then it has been renamed TXIB formulation additive.

TXIB



(Eastman, 2009e)

Producer's description (extracts)
Eastman has given the following presentations of TXIB (Eastmann, 2006, 2009f): TXIB formulation additive is a superior primary plasticiser for PVC plastisols. It has good compatibility with PVC, and it is compatible with all common primary and secondary plasticisers. TXIB provides low viscosity characteristics in plastisols with good viscosity stability over time. TXIB is the lowest viscosity (9 cps) additive available to the flexible PVC industry. TXIB is completely compatible with PVC in all proportions and is usually blended with general-purpose plasticisers such as DOP or DOTP. The unique low viscosity makes this material particularly suitable for PVC plastisols and often allows adding additional fillers to the plastisol, resulting in a cost savings. TXIB has efficiency generally equal to DOP, which makes substitution in a vinyl formulation very easy. It imparts a dry surface to the vinyl, excellent resistance to staining, and physical properties equivalent to DOP. TXIB is also used in inks, coatings, urethane elastomers, and nail polish lacquers.

Application/Uses:

- Automotive OEM

- Coatings for automotive plastics
- Lithographic and letterpress oil-based inks
- Nail care
- Phthalate-free diluent for MEKP formulations
- Plastisols
- Sheet vinyl flooring
- Toys/Sporting goods
- Traffic cones
- Vinyl compounding
- Vinyl gloves
- Wall coverings

Application and market experience

Eastman has not wished to provide detailed feedback for this project on TXIB's suitability for substituting for DEHP, DBP and BBP. Eastman has informed that TXIB cannot be used as a direct replacement for DEHP, DBP or BBP. TXIB is used to lower the viscosity of plastisols; one function DBP has been used for. TXIB does however not increase gellation speed for faster production, as DBP and BBP is used for. TXIB cannot work as a primary plasticiser (Eastman, 2009).

In a study of plasticiser alternatives for non-PVC applications (Danish EPA, 2001a), TXIB was proposed by market actors as a substitute for phthalates in adhesives and sealants.

Key characteristics

Table 100 below shows selected comparisons from Eastman (2009c) between TXIB, DEHP ("DOP"), DEHT ("168") and other plasticisers of interest for selected parameters (more parameters are shown in reference). Note that TXIB in PVC has lower extractability in oil and hexane than DEHP, but higher in soapy water. The modulus (resistance to elongation) and low temperature flexibility is similar to DEHP, while the volatility of TXIB is a factor three higher than DEHP. Plastisol viscosities are lower than for DEHP.

Table 100. Comparison between DEHT ("168"), DEHP ("DOP"), TXIB and other plasticisers (from Eastman, 2009c)

Soapy Water Extraction (% wt loss)			Oil Extraction (% wt loss)			Hexane Extraction (% wt loss)		
Eastman Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled
TOTM	0.2	0.1	*TXIB*[f]	14	6.3	*TXIB*[f]	31	24
168	0.5	0.4	DOP	15	8.3	168	31	26
DOP	1.1	0.3	168	16	10	DOP	34	26
TXIB[f]	1.7	1.2	TOTM	24	10	TOTM	35	26
DOA	1.9	0.9	DOA	31	18	DOA	35	29

100% Modulus psi (MPa)[a]			Low-Temperature Flexibility Temperature Where T = 35,000 psi (241 MPa),[b] °C			Activated Carbon Extraction (% wt loss)[c]		
Eastman Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled	*Eastman* Plasticizer	Plastisols	Milled
DOA	800 (5.5)	1,050 (7.2)	DOA	–57	–54	TOTM	0.8	0.5
TXIB[f]	1,000 (6.9)	1,600 (11.0)	168	–38	–26	168	1.2	1.0
DOP	1,050 (7.2)	1,550 (10.7)	DOP	–38	–26	DOP	2.5	1.5
168	1,250 (8.6)	1,600 (11.0)	*TXIB*[f]	–38	–24	DOA	3.7	2.8
TOTM	1,250 (8.6)	1,850 (12.8)	TOTM	–36	–20	*TXIB*[f]	7.0	6.0

Plastisol Viscosity P (Pa·s)[d]		
Eastman Plasticizer	1 Day	21 Days
DOA	45 (4.5)	90 (9.0)
168	75 (7.5)	110 (11.0)
TXIB[f]	95 (9.5)	180 (18.0)
DOP	120 (12.0)	220 (22.0)
TOTM	190 (19.0)	265 (26.5)

Notes: Plastisols contain 60 phr plasticiser; milled and calendered PVC contains 50 phr plasticiser; phr meaning parts per 100 parts hard PVC by weight. a) Stress at which PVC is elongated 100%. Lower value indicates higher efficiency of plasticiser (ASTM D638). b) Temperature at which the shown stiffness (torsion) is reached (ASTM D1043). c) Indication of volatility at elevated temperatures (ASTM D1203). d) Brookfield viscosity, determined with a number 4 spindle at 6rpm and 23 C.

Table 101 below shows some other performance dataset for TXIB compared to DEHP, DBP and BBP. As shown, here TXIB has higher hardness (lower efficiency) than the phthalates, high volatility (yet lower than DBP) and much higher extractability in water. Extractability in kerosene is higher than BBP, but lower than DEHP and DBP.

Table 101. Technical key parameters of TXIB compared to DEHP, DBP and BBP (from Sears and Darby, 1982)

Plasticiser in PVC, conc. 40% =67 phr in same PVC resin	Shore A hardness	Volatility,% lost, 1 day at 87 °C over activated carbon	Extracted in water,%	Extracted in kerosene (jet fuel, etc.),%
DEHP	69	4.5	0.01	44
DBP	62	45.4	0.25	9.1
BBP	68	7.7	0.07	3.4
TXIB	76	23.7	2.83	5.2

Table 102 below describes some key characteristics of TXIB as alternative to DEHP, DBP and BBP.

Table 102. Key characteristics of TXIB as alternative to DEHP, DBP and BBP

Parameter	Value	Remarks
Efficiency (as plasticiser in PVC compared to DEHP)*1		Close to 1 (Eastman, 2009f)
Price (primo 2009)	NA	
Price relative to DEHP	NA	
Effective price relative to DEHP	NA	
Compatibility/solubility in PVC		Compatible
Permanency (migration, evaporation, extraction)		High volatility, see below (Wilson, 1995)
Processability (fusing speed and temperature, etc.)		Gives low viscosity to plastisols (Eastman, 2009f)
Limitations in use, if any, noted by supplier in data for this study		NA

Notes: *1: Efficiency indicator, also called substitution factor, indicating the concentration of plasticiser in PVC needed, compared to DEHP, to achieve a specified flexibility according to a well defined method. DEHP has substitution factor 1 per definition, lower = more effective, higher = less effective. NA = not available

According to Wilson (1995), TXIB shows unique performance parameters in PVC plastisols. It is a useful component of plastisols formulated to give hard end products since it confers little viscosity at low levels of addition. It has been widely used in cushion vinyl flooring for this purpose, usually in conjunction with BBP; this use has however declined greatly in the 1990s as its high volatility causes unacceptable emissions from end products.

C.12.5 Other informations on TXIB

Jansen & Bremmer (2009) made a risk assessment on the use of TXIB in toys and childcare articles. The risk assessment was based on the migration of TXIB in artificial saliva, based on a child of 8 kg (10 months) mouthing a toy for 3 hours per day. The risk assessment showed a margin of safety of 580, and normally a margin of safety of 100 is accepted. This risk assessment indicates that TXIB can be used safely in toys.

C.12.6 Conclusions on TXIB

TXIB seems to have some technically relevant characteristics as plasticiser. TXIB was found in many products analysed, 25% and 11% respectively, in two European studies of large samples of toys and childcare articles (its presence need not have been as a primary plasticiser). Given the fact that the producer does not consider TXIB an alternative to DEHP, DBP or BBP, and the information that the usage of TXIB in vinyl flooring has declined in the 1990s due to high emissions from end products, it seems that TXIB should maybe not be seen as a suitable alternative to any of these substances.

C. 13 Assessment of DIDP

DINP has been risk assessed in EU and most of the data on human health are from the EU risk assessment report from 2003 (EU RAR, 2003b).

DIDP is not a single substance but consists of two di “isodecyl” phthalate products (hereafter referred to as DIDP). There are two different CAS numbers (CAS Number: 68515-49-1 and 26761-40-0). Following information from the European Council for Plasticisers & Intermediates, these two products are prepared essentially from the same feed, through an identical olefin oligomerisation process and through similar alcohol manufacturing and phthalate esterification processes. The two phthalates are therefore considered fully

interchangeable within their whole range of the market end-uses. The substance is not classified.

C.13.1 Availability of the DIDP

DIDP is a widely used phthalate and is now used as an alternative to many applications where DEHP were used earlier often together with DINP.

C.13.2 Human Health risks related to DIDP

C.13.2.1 Toxicokinetics
Via dermal route, absorption is very low (most of the unabsorbed dose remained at the skin area at day 7). DIDP showed a very slow excretion, reflecting a slow dermal uptake process. Inhaled DIDP aerosol seems readily absorbed. It can be assumed that a part of insoluble particles are cleared from the nasopharyngeal region and swallowed.

In tissues, DIDP is mainly recovered in GIT, liver, kidneys, by oral or inhalation route, whereas following dermal exposure, muscle and adipose tissue contain most of the dose remaining in the body. Following inhalation, DIDP content in fat tissue is very low, but remains constant from the end of exposure to the end of the observation period (72 hours).

No parent DIDP or monoisodecyl phthalate (MIDP) but only metabolites (the oxidative monoester derivative and phthalic acid) are excreted in urine. In bile, DIDP was not detected in extracts 24 and 72 hours following dosing. The data on end products suggest a cleavage to the monoester and an alcohol moiety, indicating a metabolic scheme comparable to the one reported for DEHP.

DIDP is rapidly eliminated and not accumulated in tissues, less than 1% of the radioactivity was recovered in tissues after 72 hours. By oral and inhalation routes, excretion is shared between urine and faeces. By dermal exposure, only faecal elimination was indicated, but considering the low rate of recovery and by analogy with the two other routes and with the DINP behaviour, the same scheme may be anticipated. In addition, results from the two-generation study suggest a possible transfer of DIDP through the milk when dams are exposed by oral route.

C.13.2.2 Acute toxicity
It is concluded in the Risk assessment report that DIDP has a low acute toxicity by all routes of administration. In an inhalation study, there was no systemic effect observed and toxicity was limited to local inflammatory changes in the lung.

C.13.2.3 Irritation
Human or animal data suggest no potential irritant effects on skin, eyes or respiratory system

C.13.2.4 Sensitisation
There is no evidence for skin or respiratory sensitisation.

C.13.2.5 Repeated dose toxicity
The liver was identified as a target organ as a result of oral repeated exposure. In rodents, increases in liver weight were accompanied by biochemical evidence of peroxisomal proliferation; thus, a NOAEL of 60 mg/kg/d was identified in rats from a standard 90-day study. Findings in dogs were qualitatively consistent (increases in liver weight and swollen

and vacuolated hepatocytes); a NOAEL of 15 mg/kg/d was derived from a 13-week oral dog study, in spite of the large limitations of this study.

Increases in kidney weights are also observed in repeated dose toxicity tests but in a nonconsistent way and with no concurrent histopathological changes. Renal damages are only observed in a two-generation study (about 12 weeks) from 100 - 200 mg/kg/d, but only in male rats; a specific male rat effect is generally assumed.

C.13.2.6 Mutagenicity and carcinogenicity

DIDP is not mutagenic *in vitro* in bacterial mutation assays (with and without metabolic activation) and is negative in a mouse lymphoma assay. It is not clastogenic in a mouse micronucleus assay *in vivo*. This indicates that DIDP is a non-genotoxic agent.

Regarding carcinogenicity, cell transformation tests were conducted on DIDP. One positive result obtained is in accordance with those obtained with well-known peroxisome proliferators. No carcinogenicity long-term study is available for DIDP but an increase in incidence of hepatocellular tumours in rats related to peroxisome proliferation might be anticipated, in regard with the increased incidence in tumour liver cells observed with DEHP and DINP in carcinogenicity studies.

It is well-accepted that peroxisome proliferation is specific to rodents. It has been established that peroxisome proliferators exhibit their pleiotropic effects due to activation of PPARα (peroxisome proliferator-activated receptor α) and that PPARα is expressed only at low level in humans, explaining the absence of significant response in humans to the action of peroxisome proliferators. Thus, there is no concern for a potential carcinogenic effect in humans through such a mechanism.

C.13.2.7 Toxicity to reproduction

Regarding toxicity for reproduction, in 42-44 day year old (pubertal) or adult rats there is no indication of organ reproductive effects evidenced by histological observation in repeated dose toxicity studies and a two-generation study. In this two-generation study, a decrease in mean percent normal sperm was observed but of low incidence and only in P1 generation. In pups (F1, F2 and in the cross fostering satellite group) decreases in testes weight and cryptorchidism in F2 high-dose offspring were observed, likely due to the low body weight, since no histopathological damages were observed in adult testes. There were no changes in reproductive indices. From those assays, no adverse effects on fertility may be anticipated.

Regarding developmental effects, treatment of dams from gd 6-15 did not cause structural malformations but consistently demonstrated skeletal variations (increased foetal cervical and lumbar ribs) at 1,000 mg/kg/d concurrently with slight signs of maternal toxicity and lead to a NOAEL of 500 mg/kg/d; in a two-generation rat study, body weight decrease was observed in offspring partly related to lactation at the highest dose of 0.8% and leads to a NOAEL of 0.4% (253 to 761 mg/kg/d seeing that received doses are widely dependent on the period considered).

Developmental toxicity was observed consistently in the two-generation studies, where decrease in survival indices leads to a NOAEL of 0.06% (33 mg/kg/d DIDP). A prenatal exposure study in mice conducted at 9,650 mg/kg/d does not affect pregnancy outcome, however, as this test was drawn for screening purpose, it is insufficient to conclude to an absence of effect.

DIDP is devoid of estrogenic activity *in vitro*, it shows no ability of binding to rodent or human estrogen receptors or to induce estrogen receptors-mediated gene expression. *In vivo* assays demonstrated that DIDP does not increase uterine wet weight or does not give rise to vaginal epithelial cell cornification. In a two-generation study, developmental landmarks (anogenital distance, nipple retention and preputial separation) are not impaired; this suggests a lack of anti-androgenic activities.

C.13.3 Environmental risk related to DIDP

As DIDP is an isomeric mixture, the fate and behaviour of the substance cannot be determined with accuracy. Each component of the mixture would tend to have different characteristics concerning its fate and behaviour in the environment. Nevertheless, an overall picture can be drawn, as presented below.

The major characteristics of DIDP relevant for the exposure assessment are:

- no hydrolysis in water,
- readily degradable but failing the 10-day window criterion; (based on results from simulation tests performed with diethylhexyl phthalate (DEHP), representative half-lives in surface water, soil and sediment of respectively 50, 300 and 3,000 days could be estimated for DIDP),
- an estimated atmospheric half-life of 0.6 day.

The high log K_{ow} values imply a high potential for bioaccumulation, strong sorption to sewage sludge, soils and sediments and very low mobility in soil (K_{oc} values of 111,000-611,000 l/kg). Bioconcentration factors (whole body values ranging from <14.4 to 4,000) have been reported with certain freshwater organisms.

Acute toxicity tests have been performed with several fish and invertebrate species. No effects were seen at the concentrations up to and above the solubility limit of the substance. No long-term test results with fish exposed via the water phase are available, but a two-generation feeding study has been carried out with *Oryzias latipes*, in which no impact on any populational parameter was observed. Apart from physical effects (e.g. entrapment), no effects were seen in reproduction studies with *Daphnia magna*. Furthermore, no impact on the growth of algae was observed in several species up to and beyond the solubility limit of DIDP. Similarly, no inhibition of the respiration of activated sludge was observed.

Several laboratory assays were performed on sediment dwellers, showing no effects up to the highest tested concentrations (3,000 – 10,000 mg/kg dw). Furthermore, the hatching and development of frog eggs in contact with sediment containing DIDP up to concentrations of 600 mg/kg dw was not affected.

Potential for endocrine disruption have been investigated. The most relevant test result is from the multigeneration study with *Oryzias latipes*. There were no statistically significant changes in mortality or fecundity between the treatment groups. There was no reduced egg production. Evaluation of F1 and F2 embryos showed normal development. The male to female ratios (3:1) in all groups were similar. Phenotypic gender classification of male and female fish was histopathologically confirmed to be 100% correct. Ale somatic gonadal index and liver somatic index were not significantly different in any group. Based on these data there does not appear to be an impact on any populational parameter from chronic exposure to DIDP on fish.

Short-term tests were performed with plants and earthworms. No effects were observed up to a concentration of 10,000 mg/kg dw. An assessment factor of 100 is applied instead of 1,000 as no LOECs could be determined, resulting in a $PNEC_{soil}$ of 100,000 µg/kg dw.

C.13.4 Technical feasibility of DIDP

Production, consumption volumes and uses
Approximately 95% of DIDP are used in PVC as a plasticiser. The remaining 5% are used in non-PVC applications. More than half of the DIDP used in non-PVC applications involves polymer-related uses (e.g. rubbers). The typical content of DIDP in flexible PVC products is between 25 and 50%. Approximately 9,000 t/a of DIDP are consumed in Europe in non-PVC applications. The non-PVC applications of phthalates are very small when compared to the PVC application. Non-PVC applications are in other vinyl resins than PVC, cellulose ester plastics and other polymer containing products, such as pressure sensitive adhesives and printing inks. Otherwise DIDP is applied in non-polymer applications, such as anti-corrosion and anti-fouling paints

The DIDP consumption corresponds to 22% of the total European phthalate consumption used as plasticisers. The plasticiser consumption of phthalates in Europe is about 970,000 t/a. DIDP consumption in PVC amounts to about 200,000 t/a (1997 figures).

C.14 Human health and environmental assessment of the four phthalates DEHP, DBP, BBP and DIBP

C. 14.1 DEHP

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6

C.X.X Human health risks related to DEHP

The following are from the Risk Assessment Report (EU RAR, 2008a).

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 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 C3B6F1 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 (14C) 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) where as 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 nonhuman 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.

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.

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/m3 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.

Reprotoxicity – see Annex 2

Acute toxicity

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/m3) (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 LC50 of DEHP via inhalation was in this study found to be in excess of 10,620 mg/m3 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 LD50 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 was 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 LD50 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 ("LD50 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 LD50 was estimated to be greater than 9,860 mg/kg bw. In the LD50 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 LD50 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 LD50 value > 20,000 mg/kg bw. No individual animal data are given in the report.

The studies on rats and mice are summarised in the table below.

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 LD50 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.

LD50-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, LD50-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.

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.

Summary of single exposure studies

In a study of good quality, the toxicity of a single dose of DEHP via inhalation to rats was inexcess of 10.62 mg/litre/4 hours.

The acute oral toxicity of DEHP has been studied in several experiments of good quality. The LD50-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.

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

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).

Carcinogenicity

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.

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.

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.

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.

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.

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 42. Summary of data on human health.

Acute toxicity			Local effects and sensitisation		
LD_{50}, oral mg/kg bw	LD_{50}, dermal mg/kg bw	LC_{50}, inhal.. mg/m^3	Skin irritation	Eye irritation	Sensitisation
≥9,860 (mice)	24,500	10,620 (4 hours)	slightly irritating to the skin	slightly irritating to the eye.	Might provoke bronchial hyperresponsiveness. (DEHP due to the formation of MEHP)

Table 43. Summary of data on human health.

Repeat dose, genotoxicity, carcinogenicity				Reproductive toxicity		
Repeat dose, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day	NOAEL mg/kg bw/day	Reproductive toxicity	Critical endpoint
28.9	Negative	Positive	-	Table. 4.63	NOAEL: 3.7 mg/kg bw (rat) NOAEL: 8 mg/kg (human)	Table 4.62

Environment risks related to DEHP

The following are from the Risk Assessment Reporet (EU RAR, 2008a)

Biodegradation
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 (106 d for both) is not changed. The conclusion on biodegradability is used in the EUSES model to calculate realistic worst-case mineralisation rates (DT50) 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 below.

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)

Distribution

Adsorption

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.

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 70ºC has been reported from cars exposed to the sun (BUA 1986). The vapour pressure is 320 times higher at 70ºC compared to 20oC (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 $\cdot$ 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 20ºC (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.

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 (20ºC)
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: $(3.4E\text{-}5 \cdot 391) / 0.003 = 4.43\ Pa\ m^3\ mol^{-1} \rightarrow \log H = 0.65$

The results are presented in the table below

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 previous sections). This confirms the removal rate obtained in these calculations.

Bioaccumulation

Aquatic organisms

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 14C-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

Terrestrial organisms

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 14C 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 Kplant-water value can be estimated:

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

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.

Fish

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.

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.

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 (δ 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) 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.

7) 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.

8) 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.

9) 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.

10) 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.

11) 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 δ 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.

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)

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)

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)

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

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 °C
3) Static, Semistatic or Flow through
4) Measured or Nominal concentrations
5) References

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.

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.

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.

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.

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

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 °C
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)

Toxicity to algae and higher plants

The toxicity data of DEHP to algae and higher plants are summarised in the table below.

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.

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) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in °C
3) Static, Semistatic or Flow through
4) Measured or Nominal 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

Microorganisms

The toxicity data of DEHP to micro-organisms are summarised in the table below.

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/lmg/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	nonylphenolethoxypro-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/cm³ 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

Study No[6]	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

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 methanogene-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 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/cm³ 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) Marine/ Brackish organism (otherwise freshwater)
2) Temperature in °C
3) Static, Semistatic or Flow through
4) Measured or Nominal 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

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 reatment 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 testspecies are exposed via water ii) those in which natural benthic communities are exposed via contaminated sediment and iii) studies with sludge from STPs.

<u>Exposure via water</u>

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 EC10 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 NOECsediment was about 0.6 mg/kg (dwt) and the corresponding LOECsediment 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 (nonsignificant) on *Pseudomonas* were also indicated in soil tests.

Terrestrial

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.

Toxicity to plants

The effect studies on plants are summarised in the tables below.

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/m³, corresponding to 897 mg/m³ 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 °C
2) Static, Semistatic or Flow through
3) Measured or Nominal concentrations
4) References: 1) Schweiger *et. al.*,1982

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

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.

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) Temperature in °C
2) Measured or Nominal 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

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:

NOEC (or $EC_{50\ standard}$) = NOEC (or $EC_{50\ experimen}$) * (0.034 /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 shortterm 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.

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/cm2 (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 oC. 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.

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.

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) Temperature in °C
2) Measured or Nominal concentrations
3) References: 1) Neuhauser *et. al.*, 1985
2) Diefenbach, 1998b
3) Jensen *et.al.*, 2001

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 PNECsoil. However, a question mark still exists regarding possible effects on microorganisms.

Table 44. Summary of environmental fate and ecotoxicity data on ASE

Environmental fate			Ecotoxicity				
Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae	Micro-organisms	Terrestr
Readily biodeg. DT_{50} value for STP of 0.029 days	BCF 840 (fish) 2,500 WWT (mussels) 2,700 WWT (amphipods) Kplant-water = 1,940 m^3/m^3	ND	NOEC 160 mg DEHP/kg food (wwt)	$NOEC_{sediment}$ > 11,000 and 780 mg/kg (dwt) respectively.	ND	NOECsediment 0.6 mg/kg (dwt) NOEC for respiration (>) 2,007 mg/l	The lowest normali unboun NOEC 130 mg dwt

ND = No Data

C.14.2 DBP

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6

C.X.X Human health risks related to DBP

The following are from the Risk Assessment Report – Dibutyl phthalate (EU RAR, 2004).

Toxicokinetics

Dibutyl phthalate is rapidly absorbed and excreted after oral administration as was demonstrated in studies in laboratory animals. Up to more than 90% of oral doses given to rats or hamsters was excreted in urine within 24-48h. Fecal excretion is low (1.0-8.2%).

Also in human oral absorption of DBP takes place. After dermal exposure of rats absorption occurred; ca. 60% of the dose was excreted in urine within 7 days. In feces ca. 12% of the dose was found. An *in vitro* study revealed slower absorption of DBP by the human skin (2.40 µg/cm2/hour) than by the rat skin (93.35 µg/cm2/hour).

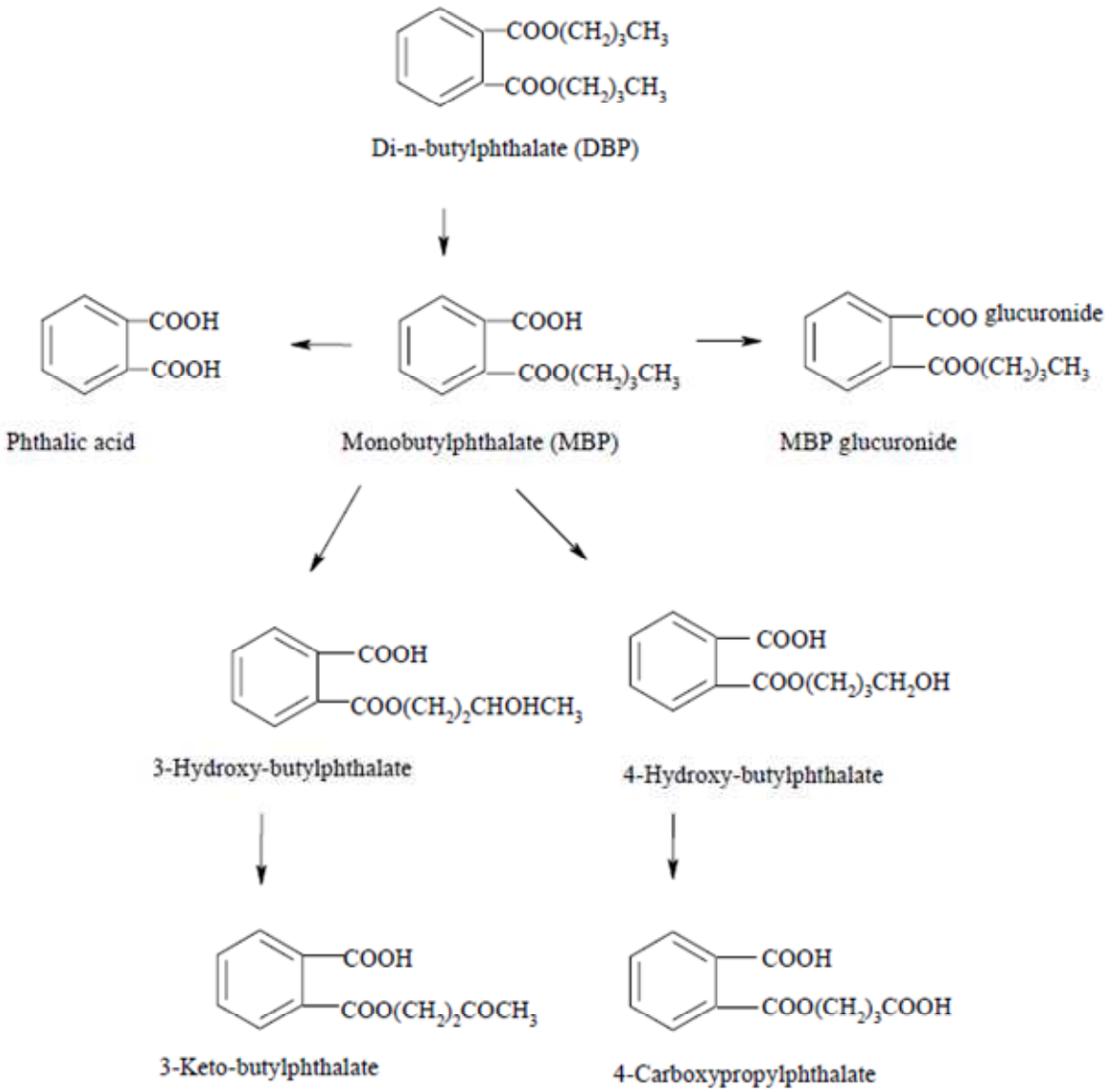
Data on absorption after exposure by inhalation are not available.

A substantial fraction of DBP is initially excreted in the bile and subsequently enters the enterohepatic circulation. No significant accumulation in tissues was observed in laboratory animals after oral as well as dermal exposure; limited inhalation data revealed an indication for some accumulation in tissues.

The major part of DBP is hydrolysed to MBP and the corresponding alcohol prior to absorption by the small intestines, but hydrolysis can also occur in liver and kidneys. The metabolites that occur in urine are MBP, MBP-glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones, carboxylates) and a small amount of free phthalic acid (see metabolism scheme below). Species differences in the excretion of MBP and its glucuronide were observed; rats excreted a larger proportion unconjugated MBP in urine than hamsters. There are no data on biotransformation after dermal exposure and exposure by inhalation.

Transplacental transfer of DBP and its metabolites was demonstrated in an oral study with 14C-labelled DBP in rats. Levels of radioactivity in placenta and embryo were 1/3 of those in maternal plasma; radioactivity in embryonic tissues accounted for less than 0.12-0.15% of the administered dose. MBP accounted for most of the radioactivity in maternal plasma, placenta and embryo. Unchanged DBP was found only in small amounts. No accumulation of radioactivity was seen in maternal or embryonic tissues.

Metabolic scheme for di-n-butyl phthalate
(Adapted from references Albro and Moore, 1974; Foster et al., 1982; Tanaka et al., 1978)



Acute toxicity

The oral LD50 value for the rat is ≥6,300 mg/kg bw for dibutyl phthalate; the dermal LD50 is >20,000 mg/kg bw for the rabbit. With respect to inhalation the 4h LC50 for dibutyl phthalate is ≥15.68 mg/L for the rat. According to the EC criteria, dibutyl phthalate does not need to be classified on the basis of its acute toxicity.

Mutagenicity

In assays detecting gene-mutations in bacteria one assay was negative in all 4 strains tested without and with metabolic activation. In two other assays equivocal and positive results, respectively were seen in strain TA 100 only, without metabolic activation. The positive effects were weak and seen at cytotoxic doses.

A gene-mutation test in yeast cells showed negative results.

In a mouse lymphoma assay performed only without metabolic activation, gene-mutations were induced at highly cytotoxic concentrations. An adequately performed test for gene-mutations in mouse lymphoma cells showed negative effects without metabolic activation;

with metabolic activation positive effects were seen. In the same experiment (Hazleton, 1986) diethyl phthalate showed negative results while it is expected that, based on structure-activity relationships, mutagenic activity would increase with decreasing length of the alkyl chain. Also butylbenzyl-,
di(2-ethylhexyl)-, diisononyl- and diisodecyl phthalate showed negative results in the same experiment.

No chromosomal aberrations in mammalian cells were seen but the tests were performed without metabolic activation only. In one test also the induction of SCE's was studied and a slight (<2x), but statistically significant increase of SCE's was seen at all three dose-levels, but without any dose-relationship.

A micronucleus study performed according to current standards showed negative results. In mice exposed for 13 weeks to DBP in their diet no induction of micronuclei was observed either. In conclusion *in vitro* studies gave an indication for a genotoxic effect in one assay, but this effect was not seen with other dialkyl phthalates in the same experiment, a.o. with diethyl phthalate. No genotoxic effects for dibutyl phthalate were observed in *in vivo* studies detecting chromosomal aberrations.

Based on the data available for dibutyl phthalate from a variety of genotoxicity studies as described above and taking into consideration the non-genotoxic properties of other phthalate esters, dibutyl phthalate can be considered as a non-genotoxic substance.

Based on the data above the substance does not need to be classified according to EC criteria.

Carcinogenicity

No adequate long-term toxicity and/or carcinogenicity studies in animals as well as humans are available.

Reprotoxicity *– see Annex 3 and 4.*

Skin irritation

Studies in animals:
In a study in rabbits performed with undiluted DBP according to OECD guideline 404, immediately after exposure and 24 hours after the start of the experiment very slight erythema was seen in 2/3 animals. Edema was not seen. 48 hours after the start of the experiment erythema had disappeared. Application area was 2.5.2.5 cm. DBP was not considered to cause skin irritation (BASF, 990a).

0.5 Ml undiluted Vestinol C (trade name of DBP) was applied to the intact and abraded skin (area 2.5.2.5 cm) of 3 m and 3 f rabbits. Per animal one intact and one abraded area was treated with Vestinol C and one intact and one abraded area with 10% laurylsulphate as positive control (FDA recommended method). Mild reactions were seen at 24 hours; at 72 hours none of the treated sites showed any reaction. Irritation index was reported to be 0.54/8. According to FDA criteria Vestinol C was very mildly irritating. According to EC criteria Vestinol C is not irritating (Greenough et al., 1981).

Studies in humans:
No data on humans are available.

Eye irritation

Studies in animals:
In a study in rabbits performed with undiluted DBP according to OECD guideline 405, welldefined conjunctival redness was seen in all animals after 1 and 24 hours, while slight to welldefined redness was seen in all animals after 48 hours. After 72 hours all symptoms had disappeared. Cornea and iris did not show irritation. DBP was considered to be not irritating for the eye in this study (BASF, 1990b).

0.1 Ml undiluted Vestinol C (trade name of DBP) was instilled in the eyes of 3 m and 3 f rabbits (FDA recommended method). The eyes were not rinsed. After 1 hour in 3/6 animals mild and in 3/6 animals very mild redness was seen. Very mild redness was still seen in 2/6 animals after 24 hours. Very mild swelling was seen in 3/6 animals after 1 hour. All eyes had returned to normal after 48 hours. No reactions in cornea or iris were seen. The irritation index was reported to be 0.11/110. DBP was considered to be not irritating for the eye in this study (Greenough et al., 1981).

Studies in humans:
No data on humans are available.

Sensitisation
Dibutyl phthalate did not show skin sensitising properties in two maximization tests in guineapigs. According to EC criteria the substance does not need to be classified on the basis of the available tests.

The results of the available case studies with respect to the possible induction of sensitisation in human by DBP are not appropriate for a definite conclusion due to the limited documentation of the studies and additionally sometimes conflicting results of the studies.

Repeated dose toxicity
An oral NOAEL of 152 mg/kg bw can be derived from a 3-month dietary study in rats performed according to current standards. A NOAEL of 19.9 mg/kg bw for peroxisomal proliferation in rats was found in a special study examining this effect. However it has to be noted that human have a relative low sensitivity for this effect.

The available studies with repeated dermal exposure are not appropriate for establishing a NOAEL.

For repeated inhalation exposure a NOAEC of 509 mg DBP/m3 (the highest concentration tested) for systemic effects including neurotoxic effects can be established based on a 28-day inhalation study in rats performed according to current standards. For local effects after repeated inhalation exposure a LOAEC of 1.18 mg/m3 can be derived from the same 28-day inhalation study.

C.3.3 Environment risks related to DBP

The following are from the Risk Assessment Report – Dibutyl phthalate

Biodegradation

The metabolic pathway of aerobic and anaerobic biodegradation of phthalates can be summarised as follows. First the di-ester is hydrolysed into the mono-ester by esterases with low substrate specificity. Subsequently the mono-ester is converted into phthalic acid. The hydrolysis of the mono-ester appears to be the crucial step which limits the rate of degradation. Further degradation differs according to the bacterial genus. There is ample evidence that DBP is ready biodegradable under aerobic conditions (a.o. ECETOC, 1985; BUA, 1987; RIVM, 1991). This is also concluded by a TemaNord report (1996), where it is stated that DBP may be considered to be readily biodegradable although several tests have been carried out under acclimated conditions. However, a BOD5:COD ratio of 0.63 obtained with a non-adapted inoculum "indicates that DBP may be regarded as readily biodegradable". Recently it was also demonstrated that DBP is readily biodegradable in a modified Sturm test (Scholz et al., 1997).

Distribution

The Henry's law constant of 0.27 Pa.m3/mol indicates that DBP will only slowly volatilize from surface waters, i.e. virtually all of the DBP will remain in the water phase at equilibrium.

The octanol/water partition coefficient (Kow) of DBP is high and consequently the equilibrium between water and organic carbon in soil or sediment will be very much in favour of the soil or sediment. Soil and sediment thus appear to be important sinks for DBP. Resuspension of DBP from the sediment to the water column may occur. Although DBP is only poorly soluble in water, it may be transported in water following the adsorption of DBP to humic substances.

Despite its low volatility, DBP has been reported as particulate and as a vapour in the atmosphere. In the air DBP is transported and removed by both wet and dry deposition.

Applying the QSAR Koc = 1.26 Kow $^{0.81}$ from the Technical Guidance Document (EC, 1996) a
Koc of 6,340 l/kg can be calculated using the log Kow of 4.57. The impact on the outcome of the risk assessment. This value is used to derive the following partition coefficients:

- Kpsusp: solids-water partition coefficient of suspended matter: 634 l/kg;
- Ksusp,water: suspended matter-water partition coefficient: 159 m3/m3;
- Ksed, water: sediment-water partition coefficient: 159 m3/m3;
- Ksoil, water: soil-water partition coefficient: 190 m3/m3.

Based on the above-cited physical chemical characteristics (log H = -0.6;) as well as the biodegradation rate of 1 h-1, the removal of DBP in the STP is estimated (EUSES) as follows:

% to air 0.07
% to water 9
% to sludge 33
% degraded 58
% removal 91

Bioaccumulation

The high Kow of DBP indicates that the substance has a potential for bioaccumulation. However, the actual degree of bioaccumulation *in vivo* will be determined by the metabolisation and the elimination rate of the substance. For phthalates it is known that an important biotransformation pathway is the formation of the mono-ester and the subsequent formation of phthalic acid. Especially the formation of the mono-ester is relevant from a

toxicological point of view since this substance has been demonstrated to cause reproductive effects in mammals.

On the other hand it should be noted that the log Kow of the mono-ester is around 2.8 which does not indicate a high potential for bioaccumulation. The available BCF data demonstrate a relatively low bioconcentration but also indicate that higher BCF values are obtained when the BCF is calculated for the total amount of metabolites using 14C-labelled material.

Reported BCFs for DBP for various organisms range from 2.9 for the brown shrimp (*Penaus aztecus*) to 2,125 for the fathead minnow (*Pimephales promelas*) (Canadian EPA, 1994). However, in both tests the criterion for bioaccumulation was the 14C-content arising from a labelled material. This may give an overestimation of the BCF due to the fact that both 14C-DBP and any 14C-labelled metabolites of DBP were measured (including 14C built into the tissue of the organism in e.g. fatty acids). The same is true for the studies of Mayer and Sanders (1973) in which very high BCFs (e.g. 5,000 and 6,700 for *Daphnia magna* and *Gammarus pseudolimnaeus*, respectively) were found. A much lower BCF of 11.7 was found in a fish study with *Cyprinidon variegatus* (Wofford et al., 1981). As in this study a static method was used, it may be an underestimation of "BCF".

Recently a bioaccumulation test according to international guidelines (OECD 305E) has been carried out under GLP by industry (Hüls, 1996). Carp (*Cyprinus carpio*) were exposed to 10 and 50 µg/l for 28 days. Based on measurements for the highest exposure concentration in water and fish a BCF value of 1.8 l/kg was found. It should be stated that this test showed some experimental shortcomings (e.g. rather weak recovery performance, unidentified background contamination and a remarkable (unclarified) drop in DBP levels during exposure period). Apart from these inconsistencies it should also be noted that also in this test the major metabolite, i.e. the mono-ester MBP, was not analysed. Hence the observed BCF only refers to the parent compound. A BCF that would include the mono-ester would probably be somewhat higher, but is expected to be lower than the BCF values measured with 14C-labelled material. The experimental BCF of 1.8 l/kg for DBP from the recent study will be used in the further risk assessment for secondary poisoning. In the risk characterisation attention will be paid to the possible consequences of using a higher value. No experimental BCF data are available for terrestrial species. EUSES calculates a BCF worm of 13kg/kg. Ray et al. (1983b) measured the concentration of DBP in marine sediment, clams and the bristle worm (Neanthes virens) from samples near Portland, Maine US. The concentrations in sediment were found to be higher than those in biota, 160 and 100 µg/kg, respectively (BCFs <1).

Toxicity

Fish
The IPCS document on DBP contains a few long-term toxicity studies with fish (IPCS/WHO, 1997). The lowest NOEC was observed in a 99-day test (60 days posthatch) with *Oncorhynchus mykiss* (Ward and Boerie, 1991). A measured value of 100 µg/l was established based on growth as the most sensitive endpoint.

Daphnia
The IPCS document on DBP (IPCS/WHO, 1997) contains some other long-term toxicity studies with aquatic invertebrates. The effect concentrations in these tests were all larger than 100 µg/l.

Algae
The test with the marine *dinoflagellate Gymnodium breve* showed a very poor reproducibility. In the first assay an EC50 of 0.0034 mg/l was established, whereas in the

second, a value of 0.2 mg/l was found. It is doubtful whether such a large difference can be attributed to biological variation.

This is supported by the fact that the variation between the replicates was much smaller in the tests with other phthalate esters in the same study. The BUA report (BUA, 1987) revealed some more, technical shortcomings of the Gymnodium test. For these reasons the test results will not be used for the derivation of the PNEC for the aquatic compartment. It is necessary to discuss the 14-day LOEC of 0.002 mg/l for the blue-green algae *Synecchococcus lividus* in more detail. At DBP concentrations of 0.002 mg/l and higher, the number of monodispersed (i.e. non-aggregated) cells of the blue-green algae was found to be significantly decreased. This effect, however, can be fully attributed to a DBP induced shift from nonaggregated towards aggregated cells. At each concentration of DBP tested namely, the percentage of aggregated *S. lividus* was found to be increased: at 0.002 mg/l about 95% of the algae were in aggregated form as opposed to 22% in the control group.

In addition, when counting the total number of *S. lividus*, i.e. both aggregated and non-aggregated organisms, a significant increase was found at all test concentrations. In conclusion it can be said that DBP caused a decrease only in the number of non-aggregated *S. lividus*. Nevertheless, very low concentrations of DBP seem to affect the growth behaviour of these blue-green algae. At present, however, the ecological significance of this effect is unknown and therefore the value will not be used for the PNEC derivation.

Microorganisms
In both *Pseudomonas putida* tests no effects of DBP were found even at concentrations above the water solubility of the substance. The low toxicity of DBP to bacteria is supported by the results of the biodegradability test (Huls study, 1995; modified Sturm test). In this test, showing ready biodegradability of DBP, a test concentration of 21.7 mg/l was used.

The MICROTOX test cannot be used for the derivation of a PNECmicroorganisms that is relevant for a STP situation, as a saltwater species is used.

Terrestrial
Invertebrates:
For the earthworm *Eisenia fetida* a 48-hour LC50 of 1.4 mg/cm2 was found in a contact test in which DBP was applied to filter paper. The toxic unit refers to the amount of chemical per cm^2 of paper (Neuhauser et al., 1986).

DBP applied to female house flies topically or by injection at a concentration of 20 μg/fly (1,000 μg/g) was not toxic, causing a mortality of less than 16% after 24 hours (Al-Badry and Knowles, 1980).

Both invertebrate tests are considered not to be useful for deriving a PNEC for the terrestrial environment.

Plants:
In a limited greenhouse experiment in which seeds of corn *Zea mays* were planted in a sandy soil containing 0 to 20,000 mg DBP/kg, germination was not affected at any concentration. After 3 weeks of exposure, plant height and shoot fresh weight were reduced significantly at 2,000 mg/kg (17% and 25%, respectively); a concentration of 200 mg/kg was without effect (NOEC). After planting a second group of seeds in the soils, plant growth was only reduced at 20,000 mg/kg, while concentrations <2,000 mg/kg were without effect. These results indicate that plant available DBP levels have decreased through complex formation with soil components and/or by degradation (Shea et al., 1982).

In laboratory tests in which spinach and pea seeds were planted in potting soil, the effects of

DBP and three other phthalate esters on plant height were investigated. No effects were found after 2 weeks of exposure to concentrations up to 1,000 mg/kg DBP. The test substance was added to the soil as aqueous solution. The effect of germination was studied with tap water to which a methanol solution of DBP was added at concentrations of 100 to 1,000 mg/l. DBP inhibited germination at 1,000 mg/l, especially that of pea seeds. No effects were found on the subsequent development of the seeds that did germinate (Herring and Bering, 1988). It should be noted, however, that this test concentration was much higher than the saturation concentration of DBP in water.

C.14.3 BBP

Group of plasticiser	Chemical name	Abbreviation	CAS no.
Sulphonates	Sulfonic acids, C10 – C18-alkane, phenylesters	ASE	91082-17-6

Human health risks related to BBP

The following are from the Risk Assessment Report – Benzyl butyl phthalate (EU RAR, 2008b).

Toxicokinetics

In rats, the kinetics of BBP after oral administration was dose-dependent. Excretion of radiolabelled BBP in the urine was between 70% and 80% in the dose-range of 2 mg/kg p.o. and 200 mg/kg p.o. whereas 22.4% were excreted in the urine after administration of 2,000 mg/kg p.o. The excretion of radioactivity in the feces was 20% after intravenous administration which indicates that the absorption in the dose range between 2 mg/kg p.o. and 200 mg/kg p.o. is nearly complete. After dermal application, 30-40% of the applied amount seems to be absorbed and reaches the systemic circulation. The extent of systemic availability of the substance administered by inhalation is not known as specific data are lacking.

BBP is metabolized to monobutyl phthalate or monobenzyl phthalate. This metabolism may take place in the gut wall and/or liver. In adult and immature rats, the ratio of monobutyl phthalate to monobenzyl phthalate found in the urine is 3:1. Both metabolites were found in the bile.

Reabsorption from gut lumen may take place. There is no evidence of tissue accumulation. The percentages of excreted metabolites (MBuP and MBeP) in the urine in adult rats were shown to be higher compared to immature rats. The excretion of BBP metabolites in urine has also been studied in humans. Contrary to the metabolism of BBP in rats, BBP is mainly metabolised to MBeP in humans, which indicates that the excretion of MBeP in the urine reflects exposure to BBP. However, limited data on the metabolism of BBP in humans is available.

No half-life of BBP in the body has been calculated. However, the available data indicate a half-life of less than 24 hours.

In the risk characterization, 100% absorption is assumed for both inhalation and oral exposure, whereas the absorption for dermal exposure is set at 5%.

Acute toxicity

The acute toxicity of BBP in animals is low. The LD_{50} values are presented in the table below. Signs of toxicity include: reduced appetite, weight loss, reduced activity, apathy, leucocytosis, collapse and death. Histological examination revealed toxic spleenitis, haemoragic areas of the lungs, liver discoloration, acute gastrointestinal inflammation and degenerative lesions of the Central Nervous System. The oral LD_{50} values of BBP ranged from 2,330 - 20,400 mg/kg bw for rats, and were 4,170 and 6,160 mg/kg bw in female and male mice. Dermal exposure of rabbits gave a LD_{50} value > 10,000 mg/kg bw. The dermal LD_{50} is in the same range as the LD_{50} values resulting from oral or i.p. administration, thus, supporting the conclusions from the dermal absorption studies with BBP. No information of exposure by inhalation is identified. The wide range of LD_{50} values in rats after oral administration of BBP (from 2,330 mg/kg to 20,400 mg/kg) may be due to the relatively low water solubility of BBP. The highest LD_{50} value was obtained when BBP was given undiluted by gavage, and the lowest LD_{50} value was obtained when BBP was administered by gavage in corn oil vehicle.

Study design	LD_{50} values	References
Oral		
Rats, Sprague-Dawley; 2-3/sex/group; Administration by gavage; 12,600, 15,300, 20,000 and 25,100 mg/kg.	20,400 mg/kg.	Hammond et al. (1987); Monsanto, (1976a)
Rats; 16-18 by average.	Killed animals at doses > 4,000 mg/kg	Mallette and Von Haam (1952)
Rats, Fisher -344; Administration by gavage.	2,330 mg/kg	NTP (1982a)
Mice, $B6C3F_1$, male and female; Administration by gavage.	Male: 6,160 mg/kg female: 4,170 mg/kg	NTP (1982a)
Intraperitoneally		
Rats; 16-18 in average.	Killed animals at doses > 1,800 mg/kg	Mallette and Von Haam (1952)
Mice, AKR/JL; 5 mice/group.	4,000 mg/kg < LD_{50} > 5,000 mg/kg.	Monsanto (1983); Robinson and Johannsen (1985)
Mice, Swiss Webster; 500 – 16,000 mg/kg.	3,160 mg/kg.	Calley et al. (1966)
Dermal		
Rats. Study poorly reported.	6,700 mg/kg	Statsek (1974)
Rabbits, New Zealand; 1-2/sex/group; 3,980, 6,310, 10,000 mg/kg.	> 10,000 mg/kg.	Hammond et al. (1987); Monsanto study Y-76-54.

Mutagenicity

BBP showed no evidence of mutagenicity in *Salmonell typhimurium* or mouse lymphoma cells.

BBP did not induce sister chromatid exchanges (SCE) or chromosomal aberrations (CA) in CHO hamster cells. BBP induced morphological transformation in Syrian hamster embryo

cells, but not in the BALB/3T3 cell transformation system. BBP did not induce sex-linked recessive lethals *in Drosophila melanogaster* or dominant lethal mutations in mice. Positive results were obtained in a mouse bone marrow test for SCE, however the responses were week, and the SCE test was not repeated. For the induction of CA conflicting results were reported when different observations times were compared. No inductions of micronucleus were reported in female rats after exposure to low doses of BBP (182.6 μg/kg bw/day during gestation and lactation). An overview of the studies is presented in the table below. Based on the available data, and according to EU criteria, BBP should not be considered a mutagen.

Study design	Results	Reference
In vitro, prokaryotes and lower eukaryotes		
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.1, 1.0, 5.0, 10.0 μl BBP/plate	Negative	Monsanto (1976b)
Salmonella typhimurium Strain TA98, TA100, TA1535, TA1537, TA1538; S9 +/-; 0.001, 0.01, 0.1, 1.0, 5.0, 10.0 μl BBP/plate	Negative	Monsanto (1976c)
Salmonella typhimurium Strain TA98, TA100, TA1535 and TA1537; S9 +/-; 100, 333, 1,000, 3,333 and 10,000 μgBBP/plate.	Negative	NTP (1997)

Study design	Results	Reference
In vitro, Mammalian cells		
E. Coli; 30 mg/plate; mutation test.	Negative	Omori (1976), orgianl data Kurata (1975)
B. Subtilis; 30 mg/plate; repair test.	Negative	Omori (1976), orginal data Kurata (1975)
Saccharomyces cerevisiae Strain D4, S9 +/-; 0.1, 1.0, 5.0, 10.0 µl BBP/plate; mutation test.	Negative	Monsanto(1976b)
Mouse lymphoma cells L5178Y TK; S9 +/-; 0.08, 0.16, 0.32, 0.65, 1.25, 2.5 or 5.0 µl BBP/ml, insoluble at 1.25, 2.5 and 5.0 µl/ml. Mutation test	Negative	Monsanto (1977d)
Mouse lymphoma cells L5178Y TK; S9 +/-; 5, 10, 20, 30, 40, 60 nl BBP/ml. Mutation test.	Negative	NTP (1997)
Chinese hamster ovary (CHO) cell; S9 +/-; Up to 1,250 µg BBP/ml; CA and SCE assay.	Negative or ambiguous	Galloway (1987)
Syrian hamster embryo cells; 25, 50, 100, 150, 250 µg BBP/ml in the 7 days study, 1,2 5,10 and 20 µg BBP/ml in the 24 hours study, precipitation at conc. ≥ 25 µg BBP/ml. Cell transformation test.	Negative in the 24 hours study; positive at 2.5 and 10 µg/ml in the 7 days study.	Le Boeuf (1996)
BALB/3T3 cells; 10, 20, 40, 80, 160 nl BBP/ml. Transformation test.	Negative	Monsanto (1985)
In vivo		
Drosophila melanogaster; 250, 10,000 and 50,000 ppm BBP in feed; sex-linked recessive lethal mutation.	Negative	Valencia (1985)
Mouse bone marrow; 1,250, 2,500 and 5,000 mg BBP/kg bw i.p; SCE and CA assay.	SCE weak positive after 23 and 42 hours, CA positive at 17 hours at highest dose, negative all doses at 36 hours.	NTP (1997)
Alpk:APfSD (AP) rats. 182.6 µg/kg/day of BBP during gestation and lactation; 19 rats; micronucleus	No induction of micronucleus	Ashby et al. (1997)
B6C3F1 mice, CD-1 mice; 400-600, 1,280-1,840, 3,200-4,560 mg BBP/kg bw, s.c.; dominant lethal mutations.	No increase in foetal deaths.	Bishop (1987)

Carcinogenicity

Butyl benzyl phthalate was tested for carcinogenicity by oral administration in one experiment in mice and in three experiments with rats, including a dietary restriction study. No increases in the incidence of tumours were observed in mice. The results from the rat studies are summarised in the table below. An increased incidence of mononuclear cell leukemias was reported in female rats at 12,000 ppm BBP. The increase was within the historical controls and frequency was actually similar to the frequencies found in the two control groups in the second experiment. No significant increase in the incidence of mononuclear cell leukemias was, however, found in two later studies with the same rat strain although a higher concentration was tested. An increased incidence of benign pancreatic tumours was seen at the highest dose in one conventional study in male rats, but not at a two times higher dose after dietary restriction. A marginally increased incidence of pancreatic adenomas occurred in female rats in a convential study ($p = 0.49$), but not after dietary restriction. Pappilomas of the urinary bladder was marginally increased in female rats both in the conventionally study ($p = 0.49$) and after dietary restriction. Moreover, after dietary restriction and 32 months a non-significant ($p = 0.12$) increase in bladder carcinomas was found. The latter results are difficult to interpret as no historic controls are available. In one

study in rats, butyl benzyl phthalate given prior to 7,12-dimethylbenz(a)antracene inhibited mammary carcinogenesis. BBP may be a borderline case between no classification for carcinogenicity and Carc. Cat. 3. However, due to the lack of genotoxic effects no classification is proposed.

	NTP 1982			NTP 1997					NTP 1999 Dietary restriction			
									24 months		32 months	
	0 ppm	6,000 ppm	12,000 ppm	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	24,000 ppm	0 ppm	24,000 ppm*	0 ppm	24,000 ppm*
Male												
Pancreas: adenoma	Terminated. after 30 weeks			3/50 (6%)	2/49 (4%)	3/50 (6%)	**10/50 (20%)**		0/50 (0%)	0/51 (0%)	0/50 (0%)	3/41 (6%)
Female												
Pancreas adenoma	0/47 (0%)	3/46 (7%)	0/46 (0%)	0/50 (0%)		0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Mononuclear cell leukaemia	7/49 (14%)	7/49 (14%)	**18/50 (36%)**	21/50 (42%)		20/50 (40%)**	21/50 (42%)	19/50 (38%)	16/50 (32%)	18/50 (36%)	29/50 (58%)	39/50 (78%)
Bladder, papilloma	No data			1/50 (2%)		0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	2/49 (4%)	1/50 (2%)	2/50 (4%)
Carcinoma	No data			0/50 (0%)		0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	**4/50 (8%)**

Bold indicates significant increase compared to control.
* 12,000 ppm for males.
** The historical controls for mononuclear cell leukaemia varies today from 16% to 42% with an average of 29.3% (NTP, 2000)

Toxicity for reproduction – See section B.4.1.2.9

Irritation

In two old skin irritation studies BBP was reported to have a moderate irritating effect on animals. In the first study little information was available, and in the second study the exposure route was intradermal, an exposure route which is not considered relevant for humans. The studies are therefore not used in the risk characterisation or classification according to EU criteria. In a well conducted Draize study no irritating effect was reported in rabbit skin. In an ear swelling test no irritating potential of BBP was observed. In humans BBP was found to have no skin irritating effect. The eye irritating potential of BBP was studied in rabbits using the Draize procedures, and a slight eye irritation was reported. No human experience indicating eye irritation due to BBP exposure was located. Based on the available data, and according to EU criteria, BBP does not need to be classified as an irritant to skin or eye.

Study design	Critical effects	References
Skin		
Rabbits; 2-4 animals; Patch test; 100% BBP conentration.	Moderatly irritating.	Mallette and Von Haam (1952)
Rabbits; Intradermal injection (0.2 ml); 100 mg/ml BBP.	10 min no 15 min mild 26 min moderate	Calley et al. (1966)
Rabbits, New Zealand; 6/ group, 2 groups; Draize skin test; 0.5 ml BBP.	Negative.	Hammond et al. (1987); Monsanto (1976a)
Mouse, AKR/JL, epicutaneously injection in ear, 1.44, 14.4, 144 and 1,440 mM.	Negative.	Monsanto (1983)
Humans; 200 human volunteers; patch test; 0.2 ml/patch.	Negative.	Monsanto (1980); Hammond et al. (1987)
Humans; 15-30 humans; 10% concentration. Of BBP; patch test.	Slightly irritating in 12% of the human tested.	Mallette and Von Haam (1952)
Eye		
Rabbits, New Zealand; 6/group, 2 groups; 0.1 ml of BBP.	Slight degree of irritation.	Hammond et al. (1987); Monsanto (1976a)

Sensitisation

In an old study BBP had a slight skin sensitasing effect in rabbits. It appears that BBP was negative in the various studies performed to assess a skin sensitisation potential of BBP using the ear swelling test in mice and guinea pigs. However, the test has not been fully evaluated and no standard protocols are available. Equivocal results were obtained when BBP was tested for antibody formation in mice. Furthermore, BBP did not form hapten-protein complexes which indicate that BBP is incapable of inducing immune hypersensitivity. No skin sensitisation was reported in two human studies with BBP. In a case-control study an association was found beetween childeren exposed to BBP in house dust and cases of allergic symptoms in children. However, in this study very small differences were found in the concentrations of BBP in house dust from controls and cases of allergic symptoms. Furthermore, demographic factors and pet ownership was not considered in this study. Based on the available data and according to EU criteria BBP does not need to be classified as a sensitiser.

Repeated dose toxicity

Repeated exposure of rats to BBP resulted in decreased body weight gain, alterations in haematological parameters, and damage to the testes, epididymis, prostate, liver, kidney, spleen and pancreas. In mice and dogs the only reported effects are a reduction in body weight gain. The derived NOEL/NOAEL/NOAEC/LOAEL of the various studies is given in the table below.

Study design	Effect level	Critical effect	References
Oral			
Sprague-Dawley rats; 6 male/group; 14 days; gastric intubation; 160, 480, 1,600 mg/kg bw/day		At 480 mg/kg bw/day histopathologic changes in testis in 1/6 rats. At 1,600 mg/kg bw/day body weight decrease, liver enlargement and ultrastructural changes with an increase in peroxisome numbers in the liver. Testicular atrophy and decreased testis weight.	Lake et al. (1978)
Charles River CD rats; 10/sex/group; 6 weeks; Administration in diet; 500, 1,500, 3,000 mg/kg bw/day.		No evidence of neurologic impairment.	Robinson (1991)
Sprague-Dawley rats; 5-10/sex/group; 4 weeks; Administration in diet; 500, 1,000, 1,500, 2,000, 3,000 and 4,000 mg/kg bw/day	NOAEL: 1,000 mg/kg bw/day	At doses ≥ 1,500 mg/kg bw/day body weight decrease. From 1,500 mg/kg bw/day testicular atrophy. From 2,000 mg/kg bw/day stiffness while walking and bleeding around nares.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 2 weeks; gastric intubation; 160, 480 and 1,600 mg/kg bw/day		At 1,600 mg/kg bw/day body weight decrease and testes atrophy.	Hammond et al. (1987)
Cpb-WU male rats; 4 weeks of age; Administration of BBP by gavage 28 days; 3/group; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600, 2,100 mg/kg bw/day.	NOAEL for systemic toxicity (increased liver weight): 580 mg/kg bw/day	Liver weight increase from 750 mg/kg bw/day. A dose related decrease in testis weight from 750 mg/kg bw/day, however, statistically significant from 1,250 mg/kg bw/day. Severe testicular atrophy from 970 mg/kg bw/day. Decreased testosterone levels from 450 mg/kg bw/day.	Piersma et al. (2000)
Sprague-Dawley rats; 10/sex/group; 3 month; Administration in diet; 2,500 - 20,000 ppm (corresp. to approx. 188, 375, 750, 1,125, 1,500 mg/kg bw/day)	NOAEL female: 375 mg/kg bw/day NOAEL male: 750 mg/kg bw/day	At doses ≥ 750 mg/kg bw/day kidney and liver weight increase in females, at doses ≥ 1,125 mg/kg bw/day liver weight increase in males.	Hammond et al. (1987)

The most relevant oral studies in rats are two 90-day sub-chronic studies in male and female Sprague-Dawley rats and Wistar rats (Hammond et al., 1987), and a 26-week oral toxicity study in male Fisher rats (NTP, 1997). In Wistar rats a slight anaemia, a decrease in urinary pH, and slight changes in relative organ weights were reported. Histopathologic changes were limited to the liver, pancreas and testes. The NOAEL in male Wistar rats was 151 mg/kg bw/day of BBP and the LOAEL 381 mg/kg bw/day based on histopathological changes in the pancreas, gross pathological changes in the liver, increased kidney weight and decreased urinary pH. In Sprague-Dawley rats an increase in relative liver and kidney weight were observed at 750 mg/kg bw/day of BBP, whereas no compound related lesions were observed at necropsy or upon histopathologic examination. The NOAEL in Sprague-Dawley rats was 375 mg/kg bw/day of BBP. In the 26 week study in Fisher rats, anaemia, decreased testes weight, hypospermia and atrophy of the seminiferous tubules were reported at 1,600 mg/kg bw/day, and at 550 mg/kg bw decreased liver weight and increased cell haemoglobin concentrations were reported. At 180 and 550 mg/kg bw/day minimal erytrocyte count occurred sporadically. The NOAEL in Fisher rats was 180 mg/kg bw/day, and the LOAEL 550 mg/kg bw/day. In the 3 month oral study in Beagle dogs the only effect reported was a decrease in body weight at 1,852 mg/kg bw/day. The effect reported in dogs at relative high concentrations of BBP compared to effects of BBP reported in mice and rats could be due to pharmacokinetic differences. In beagle dogs approximately 90% of unchanged BBP was measured in faeces over a 4 hours period (Erickson, 1965, see Section 4.1.2.1).

The most relevant inhalation study is a 90-day sub-chronic study with male and female Sprague-Dawley rats performed according to current Guidelines and GLP. Relative organ weight changes were observed in the liver and kidney in both sexes at 789 mg/m3 of BBP, and a decrease in serum glucose. At 218 mg/m3 an increase in kidney weight was reported in male rats at interim sacrifice. However, no compound related macroscopic or microscopic lesions were observed. The NOAEC from the study was 218 mg/m3 of BBP, and the LOAEC 789 mg/m3 based on increased kidney and liver weight in male and female rats.

BBP has been shown to cause peroxisom proliferation in both male and female rats. There was no obvious sex difference in the induction of peroxisome proliferation based on the 21-day feeding study by Barber et al. (1987). Compared with DEHP, BBP appears to be somewhat less effective in causing peroxisome proliferation, at least in male rats. Peroxisome proliferation was in all studies measured as an increase in PCoA and/or CAT activity as an index for peroxisome proliferation or lauric acid 11- and 12-hydroxylation. The most relevant study is the 21-day oral study in male and female rats performed according to GLP. Dose-dependent increases in liver weight, hepatic PCoA as well as lauric acid 11- and 12-hydroxylation were reported after exposure to 0.6, 1.2 or 2.5% of BBP in the diet. In the 2.5% group a moderate increase in peroxisome numbers measured by electron microscopy (EM) were evident. The LOAEL from this study was 0.6% of BBP in the diet corresponding to 639 mg/kg/day of BBP, and this LOAEL value correlate well with the NOAEL and LOAEL values reported from a 28-day oral study and a 12-month oral study in rats.

In the risk characterisation for BBP for repeated dose toxicity a NOAEL at 151 mg/kg bw/day from a 90-day oral study in Wistar rats is used (Hammond et al., 1987). This NOAEL value is based on histopathological changes in the pancreas, and gross pathological changes in the liver from 381 mg/kg bw/day. In the other repeated dose toxicity studies with a duration of three month or longer the NOAEL values are based on organ weight changes (Hammond et al., 1987, Sprague-Dawley rats), decreased body weight (NTP, 1982), or slight, but statistically significant changes in haematological parameters (NTP, 1997). For inhalation exposure to BBP a NOAEC at 218 mg/m3 Monsanto, 1982) from a 13-week inhalation study in Sprague Dawley rats is considered used. This NOAEC value is based on a significant increase in absolute and/or relative kidney or liver weight at 789 mg/m3. No NOAEL value is derived for dermal exposure to BBP due to only one poorly reported study available.

The results from the repeated dose toxicity studies indicate that no classification for repeated dose toxicity (R48) is warranted.

C.3.3 Environment risks related to BBP

The following are from the Risk Assessment Report – Benzyl butyl phthalate (EU RAR, 2008b).

Biodegradation

Based on the available data on aerobic biodegradation, BBP must be considered readily biodegradable meeting the 10-day window criterion. Anaerobic degradation studies of BBP show that primary biodegradation of BBP takes place, however, with variable half lives and with a possible build-up of metabolites, mainly monoesters and phthalic acid. The results also suggest that BBP may undergo ultimate biodegradation under anaerobic conditions in sludge and sediment. However, the soil and sediment studies are not thought to be performed in a representative manner. Therefore sediment degradation rates for use in this risk assessment

are derived by estimation from the ready biodegradation test as described in the TGD. The rate constants are presented in the table below.

	[d^{-1}] at 120°C
Total rate constant for degradation in STP	24
Total rate constant for degradation in surface water	0.0462
Total rate constant for degradation in sediment	0.00023
Total rate constant for degradation in soil	0.023

Distribution

BBP is reported to have an adsorptive character to soil and sludge. A soil adsorption coefficient (K_d) of 68-350 and a sludge concentration factor of 244 have been reported by Gledhill et al. (1980) and Petrasek et al. (1983). The latter authors also observed 96% removal of BBP in a STP imulation test and attributed this to sludge adsorption (elimination). Shelton et al. (1984) reported that BBP was found in different digested sludges, indicating that complete degradation may not occur within the retention times of some municipal sludge digesters.

The high log K_{ow} and relatively low water solubility of BBP indicates a relatively low mobility in soil. However, binding of BBP to colloidal matter and humic substances may enhance subsurface transport through cracks and macropores in soils.

The table below summarises parameters relevant for estimating mobility. Comparing the values with those calculated by EUSES indicate that the EUSES estimated values are intermediate of those measured. Therefore EUSES calculated Koc value of 10,500 l/kg and Kd of 210 have been used
in this RA.

Test parameters	Test result	Reference
Octanol /water partition coefficient (log K_{ow})	4.84	Mean value of RA
Organic carbon/water part. coefficient (K_{oc}) [l/kg]	9,000 17,000 10,500	Staples et al. (1997) Russel and McDuffie (1986) EUSES (used in this RA)
Soil adsorption coefficient (K_d)	68-350 210	Measured, Gledhill et al. (1980) EUSES (used in this RA)
Sludge concentration factor[1]	244	Petrasek et al. (1983)
Concentration factor [2]	172	Patterson and Kodukala (1981)

1) Concentration measured in the sludge samples [µg/l] divided by the influent concentration
2) Sludge (wet weight) pollutant concentration divided by influent wastewater pollutant concentration [µg/l].

Bioaccumulation

The measured bioconcentration factors (BCF) based on total radioactivity are in the range 135-663 l/kg (see the table below).

Species	Conc. [mg/l]	Exposure conditions	BCF [l/kg]	Test method	Remark	Reference
Lepomis macrochirus	0.00296	17 days 22°C	BCF_{wh}=188 Parent compound and metabolites		GLP=no data BCF_{muskle}= 28 $BCF_{viscera}$= 1,693 Uptake =143 d^{-1} Depuration $t_{1/2}$= 0.75 days	Heidolph and Gledhill (1979)
Lepomis macrochirus	0.034 parent compound	3 days 22°C	BCF_{wh}= 449 ** Parent compound and metabolites (12)*	EPA 560/6-82-002	GLP=yes Total (^{14}C)metabolite BCF_{muskle}=45 (1)* $BCF_{viscera}$=684 (19)*	Carr (1992)
Lepomis macrochirus	0.00973	21 days 16°C	BCF_{wh}=663 Parent compound and metabolites	Mount and Brungs (1967)	GLP=no data Depuration $t_{1/2}$= 1-2 days	Barrows et al. (1980)
Crassostrea virginica	0.012	11 days 19.5°C	BCF_{wh}=135 Parent compound and metabolites	Mount and Brungs (1967)	GLP=yes Depuration $t_{1/2}$= 1-2 days	Springborn Laboratories (1986)

BCF Bioconcentration factor
BCF_{wh} Bioconcentration factor for whole fish
* Results in parentheses are BCF for parent compound (BBP)
** See previous page for calculation (equation 1)

The BCF-value of 12 l/kg, taking only into account the accumulation of the parent compound, would mean that BBP is not considered to biomagnify. Based on the data from Section 4 it cannot be excluded that the metabolites can give endocrine/reproductive toxicity effects to other species like birds, fish etc, as they do to mammals. However, no information on such effects in these species is currently available. Therefore the BCF-value used should cover the BCF of the parent compound (BBP) and the accumulation of the two monoester metabolites (MBuP and MBeP).

Since none of the BCF tests performed include analysis of the monoesters, the values given in total ^{14}C-labelled BCF-values will probably be an overestimation of the true BCF for BBP and the accumulation of the toxic mono-esters. The GLP study performed by Carr (1992) shows that BBP is rapidly metabolised and excreted after exposure of fish at 22°C. However chronic exposure would lead to chronic levels of monoesters that may have harmful effects. This risk assessment should also cover this risk. Based on the evaluation of the BCF-tests regarding ^{14}C-method, the experimental BCF-value of 449 l/kg for fish will be used for estimating secondary poisoning in EUSES.

Fish

The lowest acute LC_{50} is 0.51 mg/l for *Cymatogaster aggregata* (Ozretich et al., 1983). This value is relevant for classification purposes. With respect to chronic tests the NOEC value of 0.14 mg/l from the study with *Pimephales promelas* is the lowest value (EG&G Bionomics, 1981). One should also note that as a sub lethal effect in the acute test of *C. aggregata*

schooling behaviour was affected at concentrations down to 0.27 mg/l after 3 hours and down to 0.08 mg/l after 96 hours.

Daphnia

The lowest acute LC_{50} in a good quality study is 0.9 mg/l for *Mysidopsis bahia* (EG&G Bionomics BP-79-4-38). The lowest chronic NOEC equal to 0.075 mg/l is that of the *Mysidopsis bahia* test of Monsanto 86-7-2074. This NOEC value will be used in the risk assessment to derive a $PNEC_{aquatic}$.

Algae

The most sensitive species in a valid test are *Navicula pelliculosa* (Carolina Ecotox 14-01-1) with respect to both EC_{50} and EC_{10}. The E_rC_{10} value of 0.20 mg/l based on growth rate is used to derive the $PNEC_{aquatic}$. The EC_{50} value of 0.64 mg/l for *Navicula pelliculosa* may be used for classification purposes.

Microorganisms

In a respiration inhibition test (OECD 209) no effect was observed on respiratory activity in activated sludge at BBP's solubility limit of 2.8 mg/l (Volskay and Grady, 1988).

Terrestrial

The only submitted terrestrial test is an earthworm test (Huntingdon Life Science, SLU 001/983882, 1998). An acute toxicity test of BBP with earthworm (*Eisenia foetida*) was performed according to OECD 207 "Earthworm, acute toxicity test" and Directive 87/302/EEC part C "Toxicity for earthworm: artificial soil test". The study was a GLP study. The earthworm species *Eisenia fetida* (or the similar *E. andrei*) was exposed in artificial soil to BBP. Five concentrations were tested and the nominal concentrations were 95, 171, 309, 556 and 1,000 mg/kg dwt. There were four replicates with 10 worms in each replicate. Weight and survival were registered after 7 and 14 days. Neither mortality related to the chemical treatment nor differences between negative control and treatment were registered. A positive control showed an expected response. Determination of LC_{50} value is not possible due to 100% survival. The data generated in the study are considered to be valid.

C.14.4 DIBP

The following text is taken from "Data on manufacture, import, export, uses and releases of dibutylphthalate (DBP) as well as information on potential alternatives to its use, ECHA 2009a):

"
NICNAS (2007) have recently reviewed the toxicity of DIBP and the following review is based on their report..

DIBP appears to be readily absorbed via the dermal route. It undergoes primary metabolism into the hydrolytic monoester, MIBP, before excretion. Urine was the major route of excretion with minor biliary excretion being observed. There was little accumulation in the rat tissues.

Acute toxicity - DIBP has a low order of acute toxicity by the oral, intraperitoneal and dermal route.

Skin irritation - DIBP is reported to cause minimal skin irritation in guinea pigs. No eye irritation or skin sensitisation has been observed in animals but the available information is limited.

A 4-month repeated dose toxicity study reported low body and testes weights and increased liver weights in rats with a 5% diet (doses in mg/kg bw not provided in source study). The NOAEL was 1% in diet. DIBP was not mutagenic in bacterial mutation assays but there is evidence that it induced DNA damage in human cells in vitro. No in vitro chromosomal aberrations, mammalian mutation and in vivo genotoxicity studies are available. Overall, the genotoxic potential of DIBP cannot be determined.

No carcinogenicity data are available for DIBP. Due to insufficient testing on other phthalates, it is not possible to extrapolate carcinogenic potential for DIBP.

Reproductive toxicity - With respect to reproductive toxicity, DIBP induced decreased body weight after 1 week oral dosing in rats and mice as well as effects on testis weight and testosterone content (Oishi & Hiraga, 1980 b; c – cited by NICNAS). Relative testes weight was increased in mice and decreased in rats while testicular testosterone content was decreased in both species. Similar results were obtained when rats and mice were fed diets containing MIBP (Oishi & Hiraga, 1980 a; d cited by NICNAS). A NOAEL was not established in any of the animal studies.

Development toxicity - Limited developmental toxicity studies are available. Oral exposure to DIBP during gestation was associated with complete loss of litters at maternally toxic doses. At lower doses, DIBP induced decreased foetal weight and increased incidence of undescended testes (Saillenfait et al., 2006) and in male foetuses at term decreased testicular testosterone production ex vivo and testosterone levels in testes and plasma, decreased AGD, and induced pathological changes in the testes including clustering of small Leydig cells and vacuolisation of Sertoli cells (Borch et al., 2005 - cited by NICNAS). The NOAEL was 250 mg/kg bw/day based on decreased pup weight and increased incidence of undescended testes (Saillenfait et al., 2006 cited by NICNAS).

A recent human study (Swan et al. 2005 - cited by SCENIHR) showed urinary DIBP concentration was inversely related to anogenital index (AGI) (i.e. anogenital distance normalized for body weight) in male children. However, multiple exposures to different phthalates may have contributed to this effect. In addition, the reliability of endpoint measured, anogenital index, has not been verified in humans. Although data for DIBP are limited, the fertility and developmental effects observed are similar to those phthalates with side chain backbone of carbon side chains of 4-6 carbon atoms in length (C4-6) (NICNAS, 2007). These C4-6 phthalates previously referred to as ‘transitional’ phthalates (Phthalate Esters Panel HPV Testing Group, 2001 - cited by NICNAS) have also been associated with male reproductive (decreased in testicular testosterone production) and developmental (decreased anogenital distance and pathological changes in the testes) effects. Therefore, it could be argued that DIBP has a similar reproductive toxicity profile to ‘transitional’ (C4-6) phthalates for which reproductive and developmental effects are recognised.

The table below summarises the human health effects of DIBP

Endpoint	Value
LD50	16000-60320 mg/kgx
NOAEL mg/kg bw	
Reproductive toxicity Effects on male fertility	1000 mg/kg/day
Repeated dose Toxicity, NOAEL	1% in diet
Developmental Toxicity NOAEL	250 mg/kg/day (rat)
Genotoxicity	Insufficient data
Carcinogenicity	Insufficient data
Maternal toxicity NOAEL	250 (rat)
Critical endpoint	Developmental toxicity Dose: 250 mg/kg/day - rat
Preliminary DNELs	DNEL for critical endpoint, mg/kg/day
Workers, oral	350 mg/day Default assessment

	factors
General population, oral	175 mg/day
Workers, inhalation	35 mgm-3
General population, inhalation	8.75 mgm-3

Human health summary table

Acute	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcino-genicity***	***Fertility***	***Developmental Toxicity***
Oral Mouse: LD50 =12,800-39,520 mg/kg bw Rat: LD50 =16,000-60,320 mg/kg bw Intraperitoneal Mouse: LD50= 3990-12,800 mg/kg bw Rat: LD50 >16,00mg /kg bw	Skin irritation: minimal effects Eye irritation: negative Skin Sensitisation: negative	Rat: NOAEL = 1% in diet LOAEL = 5% ↓ body and testes weights (m) and increased liver weights (m+f)	<u>*In vitro*</u> Negative in bacterial mutation assays Positive in comet (DNA damage) assay using human mucosa (sample size = 70) <u>*In vivo*</u> No data	No data	*Male reproduction study* Rat: NOAEL= not established LOAEL = 2000 mg/kg bw/day ↓ testes weight and histology	*Developmental study* Rat: NOAEL <u>Devp</u> = 250 mg/kg bw/d LOAEL <u>Maternal</u> = 500 mg/kg <u>Devp</u> = 500 mg/kg ↓ pup weight and ↑ trans-abdominal testes migration

Environmental hazard

The table below describes the environmental hazard properties of DIBP. No EU risk assessment has been conducted for this substance.

Classification	• Classification = Repr. Cat. 2; R61 Repr. Cat. 3; R62 • R61 = May cause harm to the unborn child • R62 = Possible risk of impaired fertility
Compartment	Hazard / risk conclusions
Water	96h LC50 = 2500 – 3600 µg/l (crustacea) 730 – 1100 µg/l (fish)
Sediment	Unknown
Soil	Unknown
Atmosphere	Unknown
STP	Unknown
Secondary poisoning	Unknown
Bioaccumulation	Estimated BCF = 800
Persistence	"Phthalate esters undergo 50% ultimate degradation within 28 days in standardised aerobic biodegradation tests with sewage sludge inocula. Biodegradation is expected to be the dominant loss mechanism in surface water, soils and sediments"
Risk assessment conclusions	None identified

"

The dossier submitter has information/data on relevant endpoints from one of the registration dossiers for DIBP, but these data is not shown due to isues of confidentiality.

C.15 Summary of human health and environmental assessment of alternative plasticisers

C.15.1 Human health assessment summary

Table 103 provides an overview of the toxicological properties of the selected alternatives. None of the alternatives are classified according to the CLP Regulation

All substances have been tested for acute toxicity for at least one exposure route, sensitisation (except ASE), subchronic toxicity and mutagenicity. All substances except ASE and DINA have been tested for both reproductive and developmental toxicity.

With regard to carcinogenicity only ATBC, DEHT and DINCH have been tested in combined chronic toxicity and carcinogenicity studies. For DEGD, DGD and DEHT estrogenic activity has been tested in an uterotrophic assay without positive response.

Most data used for the evaluation are considered of good quality, i.e. studies following accepted guidelines (OECD or US EPA) or studies considered acceptable at the time they were carried out. For some of the studies little information is available to evaluate the quality. However, key information is obtained from IUCLID data sheets, USEPA or OECD HPV robust summaries. Studies evaluated under the USEPA HPV programme are considered to be reliable without restrictions or reliable with restrictions (Klimisch codes 1 and 2, and restrictions in cat. 2 generally not severe).

From the overview it can be seen that all eleven substances have low acute toxicity. With regard to local effects most substances are non-irritating to skin and eyes or only produce slight irritation which would not lead to classification. None of the tested substances show any sensitising potential.

Effects from repeated dose toxicity studies mainly include reduced body weight gain, increased organ weights (liver and/or kidney) and for some substances also changes in clinical chemistry or clinical pathology parameters. However, more serious pathological effects were not observed. Studies to evaluate the potential for reproductive/developmental toxicity primarily show toxic effects on parents and offspring resulting in effects on body weight or relative organ weights. Effects on foetal growth and development of cervical ribs were observed in prenatal development studies with DEGD and DGD. With regard to DEHT it was concluded that DEHT has a low potential to induce reproductive toxicity. TXIB showed statistically significant reproductive and developmental toxicity in a combined study (repeat dose/reproductive-developmental screening). Effects included reduced number of implantation sites, reduced mean litter weights and reduced mean number of live pubs.

Carcinogenicity has only been evaluated for three substances in combined studies with negative outcome.

Based on the available information as presented above and in the table below it can be concluded, that the alternatives are not worse as such than the four phthalates in respect to the human health endpoints, even though some are having different negative effects on some of the endpoints. The most commonly used alternative DINP is showing less effects than

DEHP as the potency for causing reprotoxic effects is much less than for DEHP and other endpoints are comparable or better than the levels for DEHP.

Table 103. Overview of toxicological properties of the four phthalates (data from RARs) and selected alternatives.

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
DEHP	*117-81-7*	*O: > 10,000 mg/kg* *I: 10,600* mg/m^3	*Possibly slight skin and eye irritant (not severe enough for a classification)* *Not sensitising*	*NOAEL: 29 mg mg/kg/day (kidney)*	*Negative (however evidence is not conclusive) (LOAEL 320 male rat)*	*Negative*	*REP 2* *NOAEL: 4,8 mg/kg/day*	
BBP	*85-68-7*	*O: > 2,300 mg/kg* *D: > 10,000* *I: ND*	Skin: No or slight irritation Eye: No or slight irritation Not sensitising	*NOAEL: 151 mg mg/kg/day (repeated dose tox rat – organ wight change, reduced weight)*	*Negative*	*Negative*	*REP 2 NOAEL: 50 mg mg/kg/day rat*	
DBP	*84-74-2*	*O: > 6,300 mg/kg* *D: > 20,000 mg/kg* *I: > 15.68 mg/l*	Skin: No or slight irritation Eye: No or slight irritation Appears to be not sensitising	*NOAEL: 2 mg mg/kg/day* *LOAEL: 52 mg/kg/day*	*No adequate long-term studies available*	*Negative*	*REP 2* *NOAEL 50 mg/kg bw*	
DIBP	*84-69-5*	*O: > 16,000 mg/kg*	*Not sufficient data*	*ND*	*Insufficient data*	*Insufficient data*	*REP 2 (250 mg/kg/day – rat)*	
ASE	91082-17-6	O: 26,380-31,650 mg/kg D: > 1,055 mg/kg I: ND	Skin: No irritation Eye: No irritation	NOAEL, 90 days: 228 mg/kg/day (m) 282.6 mg/kg/day (f) (increased kidney weight)	ND	Negative (Ames, mammalian cells) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	No reliable data.	-

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
ATBC	77-90-7	O: > 30 g/kg D: ND I: ND	Skin: No or slight irritation Eye: No or slight irritation Not sensitising	NOAEL, 90 days: 300 mg/kg/day (increased kidney weight) NOAEL, 2 years: 100 mg/kg/day (conservative) NOAEL, 13 weeks: 100 mg/kg/day (m) 300 mg/kg/day (f) (reduced body weight gain, increased liver weights, hepatic hypertrophy)	(No carcinogenicity observed in 2 year oral repeated dose toxicity study) No guideline study available. Existing study reliable with restrictions (lack of detail).	Negative (Ames, mammalian cells, *in vivo/in vitro* UDS test) Reliable (with some restrictions) guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Not considered toxic to reproduction (2-generation study) NOAEL: 100 mg/kg/day (parental, offspring) Reliable data available for both reproductive and developmental toxicity. Data for developmental toxicity lack some details.	Weak signs of neurotoxicity
COMGHA	736150-63-3	O: > 2,000 mg/kg D: ND I: ND	Skin: No irritation Eye: No irritation Not sensitising	NOAEL, 90 days: 5000 mg/kg/day	Negative according to the tests performed	Negative (Ames, chromosomal aberration test) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Negative NOAEL > 1159 mg/kg bw/day	-

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
DEGD	120-55-8	O: 4,198 mg/kg D: > 2,000 mg/kg I: > 200 mg/L	Skin: No irritation Eye: Slight irritation Not sensitising	NOAEL, 13 week: 1,000 mg/kg/day (reduced body weight gain, haemosiderosis in spleen, hepatocyte hypertrophy)	ND	Negative (Ames, mammalian cells, chromosomal aberration test) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Developmental tox: NOEL: 1,000 mg/kg/day (maternal) NOAEL: 500 mg/kg bw/day (prenatal dev.) NOEL: 250 mg/kg bw/day (foetal growth) Reproductive tox.: NOAEL: 10,000 ppm (500 mg/kg bw/day) (F_0 and F_1 parents) NOAEL: 3,300 ppm (165 mg/kg bw/day) (offspring) NOEL: 10,000 ppm (500 mg/kg bw/day) (reproductive) Reliable guideline studies with GLP for both developmental and reproductive toxicity..	No estrogenic activity up to 2,000 mg/kg/day (Endpoints: vaginal cornification and uterotrophic response)
DINP	68515-48-0 28553-12-0	"Low" according to RAR	Skin: No irritation Eye: No irritation Not sensitising	88 mg/kg/day (liver target organ) 15 mg/kg/day (spongiosis hepatis in rats)	No findings of relevance for carcinogenicity in humans in combined chronic tox. and carc. test.	Negative	742 mg//kg/day (mouse male fertility) Developmental tox rat 159 mg/kg/day	

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
DGD	27138-31-4	O: 3,914 mg/kg D: > 2,000 mg/kg I: > 200 mg/L (4h)	Skin: No irritation Eye: Slight irritation Not sensitising	NOAEL, 13 week: 1,000 mg/kg/day (reduced body weight gain, haemosiderosis in spleen, hepatic hypertrophy)	ND	Negative (Ames, mammalian cells, chromosomal aberration test) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Developmental tox: NOEL: 1,000 mg/kg/day (maternal) NOAEL: 500 mg/kg/day (prenatal dev.) NOEL: 250 mg/kg/day (foetal growth) Reproductive tox.: NOAEL: 10,000 ppm (F_0 and F_1 parents) NOAEL: 10,000 ppm (offspring) Reliable guideline studies with GLP for both developmental and reproductive toxicity..	No estrogenic activity up to 2,000 mg/kg/day (Endpoints: vaginal cornification and uterotrophic response)

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
DEHT / DOPT	6422-86-2	O: > 5,000 mg/kg D: > 19,670 mg/kg (guinea pig) I: no deaths in mice exposed to saturated vapour (4h)	Skin: Slight irritation Eye: Slight irritation Not sensitising	NOAEL, 90 days, 21 days 500 mg/kg/day (increased relative liver weight) NOEL, 104 weeks, 1500 ppm (79 mg/kg/day (m) and 102 mg/kg/day (f))	2-year study: No signs of tumorigenicity (unreviwed study, few details) 104 week: NOEL: ≥12000 ppm (666 mg/kg bw/day (m) and 901 mg/kg bw/day (f)) - highest dose tested (industry data) No data on guidelines for the studies. The industry study appears to follow established guidelines.	Negative (Ames, chromosomal aberration test) Reliable studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Developmental tox: NOEL: 458 mg/kg/day (maternal) NOEL: 747 mg/kg/day (developmental) Reproductive tox.: NOAEL: 500 - 700 mg/kg/day (m) 800 - 1,000 mg/kg/day (f) NOAEL, tox: 150 - 200 mg/kg/day (m) 250 - 300 mg/kg/day (f) (parental, offspring) Reliable guideline studies with GLP for both developmental and reproductive toxicity..	No estrogenic activity (Uterotrophic assay)
DINA	33703-08-1	O: > 5,000 mg/kg D: > 3,160 mg/kg	Skin: No irritation Eye: No irritation Not sensitising	NOAEL, 13 week (dog): 274 mg/kg/day (reduced body weight gain, increased liver weight, elevated enzyme levels, liver and kidney histopathology)	ND	Negative (Ames, mammalian cells) Reliable studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	No data	-

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
DINCH	166412-78-8	O: > 5,000 mg/kg D: > 2,000 mg/kg	Skin: Slight irritation Eye: No irritation Not sensitising	NOAEL, 28 days: 318 mg/kg/day (m) 342 mg/kg/day (f) (liver and kidney clinical chemistry) NOAEL, 90 days: 107.1 mg/kg/day (m) 389.4 mg/kg/day (f) (kidney weight changes, degenerated epithelial cells in urine (m)) NOAEL, 12 months: 40 mg/kg bw/day (m) and 200 mg/kg bw/day (f) - l(iver and kidney weight (m) changes)	No findings of relevance for carcinogenicity in humans in combined chronic tox. and carc. test. Reliable guideline study.	Negative (Ames, signs of chromosomal aberration - but considered non-genotoxic). Not found to be clastogenic or aeuploidogenic in micronucleus test. Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity and in vivo mammalian micronucleus test.	No developmental toxicity at doses up to 1,000 mg/kg/day Not considered toxic to reproduction (2-generation study up to 1,000 mg/kg/day) NOAEL, tox.: 100 mg/kg/day (F_1) Reliable guideline/combined guideline studies for both developmental (rat and rabbit) and reproductive (rat) toxicity. in	-

Name of substance	CAS No.	Acute toxicity O: oral LD_{50} D: dermal LD_{50} I: inhalation LC_{50}	Local effects / sensitisation	Subchronic / chronic	Carcinogenicity	Mutagenicity / genotoxicity	Reproductive toxicity	Other
GTA	102-76-1	O: > 2,000 mg/kg D: > 2,000 mg/kg I: > 1,721 mg/m^3	Skin: No irritation Eye: No irritation Not sensitising	Combined repeat dose /dev. screening: No effects at tested doses up to 1,000 mg/kg/day 90 days, inhalation: No signs of toxicity up to 2,220 mg/m^3	ND	Negative (Ames, mammalian cells, chromosomal aberration test, *in vivo* mouse nucleus) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity and in vivo mammalian micronucleus test.	Combined repeat dose / dev. screening: No significant adverse effect on reproductive parameters or offspring NOAEL(Dev/Repr.): 1000 mg/kg bw/day. Reliable guideline study.	-
TXIB	6846-50-0	O: > 3,200 mg/kg D: > 2,000 mg/kg I: > 5.3 mg/L (6h)	Skin: Slight irritation (guineas pigs) no irritation (rats and humans) Eye: No irritation Not sensitising	NOAEL, 103 days, rat: 0.1% in diet (slight increase in relative and absolute liver weight)	ND	Negative (Ames, mammalian cells) Reliable guideline studies for *in vitro* mammalian mutagenicity/genotoxicity. No *in vivo* studies available.	Combined repeat dose / dev. screening: NOEL,parental and F1: 750 mg/kg/day No effects on reproduction Combined study: NOAEL: repr., dev.: 276 mg/kg/day (m) 359 mg/kg/day (f) Reliable guideline studies for both developmental and reproductive toxicity..	-

C 15.2 Environmental assessment summary

Table 104 summarises the main data on environmental fate (biodegradation, bioaccumulation and mobility) and ecotoxicological effects (fish, daphnia and algae) of the eleven studied phthalate alternatives.

None of the alternatives are classified according to the CLP Regulation.

The data on effects on bacteria have been omitted in the summary table because the effects were generally negligible at relevant exposure levels, while terrestrial data were so sparse that a comparative evaluation cannot be made for these organisms anyway.

Useful fate data regarding biodegradability (in water) and bioaccumulative properties (either as BCF or log K_{OW}) are available for all alternatives while other fate data are quite variable and incomplete. With regard to ecotoxicological effect data, results from short-term tests with the base-set of organisms - fish, crustaceans and algae - exist for all eleven substances although the duration of some studies deviate from the current OECD standard. The low solubility of many of the phthalate alternatives has rendered it necessary to enhance solubility by means of organic solvents in order to be able to carry out the tests.

Overall, the data obtained are of good quality i.e. they are mostly based on studies performed according to accepted guideline procedures, and the studies have been evaluated (e.g. in the USEPA HPV robust summaries) to be reliable without restrictions or reliable with restrictions (Klimisch codes 1 and 2, and restrictions in cat. 2 generally not severe).

None of the eleven studied alternatives fulfil the criteria for being PBT or vPvB substances.

Table 104. Summary of environmental fate and ecotoxicity data on eleven selected possible alternatives to phthalate ester plasticisers and the four phthalates for comparison.

Substance	Environmental fate			Ecotoxicity		
	Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae
DEHP	Moderate to low	Log K_{OW} 7.5 BCF 2700	K_{OC} 5.2 (estimated)	No effects shown for at the level of solubility NOEC: 160 mg/kg food (wwt)	No effects shown for at the level of solubility NOEC: ND	No effects shown for at the level of solubility NOEC: 1 mg/l
BBP	Ready	Log K_{OW} 4.8 BCF (total radioactivity) 135-663 l/kg	K_{OC} 10,500	LC_{50} 0.51mg/L NOEC 0.14 mg/l	LC_{50} 0.9mg/L NOEC 0.075 mg/L	EC_{50} 0.64 mg/L NOEC/EC_{10} 0.15 mg/l
DBP	Ready	Log K_{OW} 4.6 BCF 1.8 l/kg (meas)	K_{OC} 6.3 (estimated)	LC_{50}(96)0.4-3 (7,3) mg/L (fish) NOEC 0.1 mg/L	LC_{50} 0.8 mg/L NOEC > 0.1 mg/L	ErC_{10} 0.2 mg/L growth rate NOEC: ND
DIBP	ND	Log K_{OW} 4.5 BCF 800 calc	ND	LC_{50}(96) 2.5-3.6	LC_{50} 0.7-1.1 mg/L	EC_{50} (72 h)1 mg/L NOEC 0.2 mg/L
ASE	Not readily biodegradable (31% in 28 d)	Log K_{OW} >6	ND (log K_{OW} indicates low mobility)	LC_{50} (96 h) >100 mg/L	EC_{50} (48 h) >1,000 mg/L	EC50 (72 h) >10 mg/l
ATBC	Ready	BCF = 250 (calculated)	K_{OC} = 1,800 (estimated)	LC_{50} (48 h) = 2.8 mg/L LC_{50} (168h) = 1.9 mg/L	EC_{50} (48 h) = 7.82 mg/L	EC50 (96 h) = 0.148 mg/L (calculated)
COMGHA	Ready	Log K_{OW} = 6.4	"Immobile in soil"	NOEC(LC_{10}) (96h) = 0.28 mg/L	EC_{50} (48 h) = 0.92 mg/L	EC_{50} (72h) = 106 mg/L
DEGD	Ready	BCF = 120 (calculated)	K_{OC} = 540 (calculated)	LC_{50} (96 h) = 3.9 mg/L	EC_{50} (48 h) = 6.7 mg/L	EC_{50} (72 h) = 11 mg/L
DINP	Ready	BCF = 800-4000	K_{OC} = 111,000-611,000 "Very low mobility in soil"	No effects shown for benthic or aquatic organisms at the level of solubility		
DGD	Ready Ultimately biodegradable under anaerobic conditions	Log K_{OW} = 3.9	ND	LC_{50} (96 h) = 3.7 mg/L	EC_{50} (48 h) = 19.3 mg/L	EC_{50} (72 h) = 4.9 mg/L NOEC (72 h) = 1.0 mg/L
DEHT	Inherent	BCF = 393	K_{OC} = 2,000	LC_{50} (7 d) > 0.25 mg/L NOEC (71d) ≥ 0.28 mg/L	EC_{50} (48 h) > 1.4 mg/L NOEC (21d) ≥ 0.76 mg/L	EC_{50} (72 h) > 0.86 mg/L
DINA	Ready	BCF ≥ 1,100 BCF	ND	LC_{50} (96 h) > 500 mg/L	EC_{50} (48 h) > 100 mg/L	EC_{50} (72 h) > 100 mg/L

Substance	Environmental fate			Ecotoxicity		
	Biodegradation	Bioaccumu-lation	Mobility	Fish	Daphnia	Algae
		(estimated) = 3.2		(nominal) LC_{50} (96 h) > 2.6 mg/L (measured)	NOEC (21d) > 100 mg/L	
DINCH	Not readily biodegradable (41% in 28 d)	BCF = 189	ND	LC_{50} (96 h) >100 mg/L	EC_{50} (48 h) >100 mg/L NOEC (21d) ≥0.021 mg/L	EC_{50} (72 h) >100 mg/L NOEC (72h) ≥100 mg/L
GTA	Ready	BCF = 1.3	K_{OC} = 10.5	LC_{50} (96 h) = 165 mg/L LC_{50} (14 d) > 100 mg/L	EC_{50} (48 h) = 380 mg/L NOEC (21d) = 100 mg/L	EC_{50} (72 h) > 940 mg/L NOEC (72h) = 556 mg/L
TXIB	Inherent	BCF = 5.2-31	ND	LC_{50} (96 h) = 18 mg/L	EC_{50} (48 h) >1.46 mg/L NOEC (14d) = 3.2 mg/L	EC_{50} (72h) = 8.0 mg/L NOEC = 5.3 mg/L

A comparison of the essential environmental effects of the alternatives with the effects from the 4 phthalates can be performed based on the data presented in table 102.

The biodegradation of the alternatives are generally categorised as “Ready” with a few exemptions (ASE and DINCH), whereas the biodegradation of DEHP is “low to moderate”.

The bioaccumulation factors are generally lower or for a few of the alternatives in the same order of magnitude as for the 4 phthalates in question, but still most of the substances have a BCF > 100 or logPow > 3 (or logKow > 4). Only about half of the alternatives have BCF values > 500, but the most commonly used ones have high values.

The mobility in soil is generally speaking lower for the alternatives, although it has to be mentioned that the data on this end point are rather scarce for the alternatives (as well as for BBP and DIBP).

For both DEHP and DINP (the main alternative to DEHP) there are no ecotoxicological effects shown for the substances at the level of their solubility. The 3 other phthalates (DBP, DBB and DIBP) are all showing ecotoxic effects (EC_{50}) < 10 mg/l for one or ore of the endpoints, whereas this is also true for some but not all of the alternatives (ATBC, COMGHA, DEGD, DGD, DEHT and TXIB). COMGHA is however not considered to be systemically toxic in aquatic species at the solubility concentration. The effects observed in fish and daphnia are considered a physical effect of the hydrophobic COMGHA rather than manifestations of systemic toxicity.

GTA (triacetin) appears to be easily biodegradable; it does not have bioaccumulative properties and has very moderate toxicity in the aquatic environment.

DEGD, DGD and DINA also come out rather favourable (if using the estimated BCF of 3.2 for DINA), while ATBC and COMGHA come out negatively despite their degradability because of their aquatic toxicities and bioaccumulative properties; however, COMGHA is a glyceride and therefore inherently metabolize and bioaccumulation is not expected. ASE and DINCH both have low acute toxicities to aquatic organisms but are not easily degradable and have high log K_{OW}

values. DEHT is also not easily biodegradable and is bioaccumulative but its aquatic toxicity cannot be fully evaluated based on the data available.

Based on the available information as presented above it can be concluded, that the alternatives are not worse as such than the four phthalates in respect to the environmental endpoints, even though some are having different negative effects on some of the endpoints. The most commonly used alternative DINP is showing less effects than DEHP as it is readily biodegradable and have less motility in soil and have BCF-values in the same order of magnitude as DEHP. Both substances show no ecotoxicological effects shown for the substances at the level of their solubility.

C.15.3 Health and environmental assessment overview

A simplified overview of the main toxicological and ecotoxicological properties of the evaluated substances is shown in Table 105.

In the table a rough overview of the quality and completeness of data is presented using the scoring system indicated in note 4 to the table.

Table 105. Overview of main toxicological and ecotoxicological properties for the four phthalates and their alternatives

Name of substance	CAS No.	Health					Environment			Data quality / data completeness (CMR and PBT)
		Acute, local and sens. effects (A/L/S)	Carcinogenic (C)	Mutagenic (M)	Repro-toxic (R)	Subchronic toxicity	Persistence	Bioaccumulation	Aquatic Toxicity	
							*1	*2	*3	*4

EHP	**11-81-7**	○/○/○	○	○	●	●	○	● **BCF**	○	**1/2**
DBP	**84-74-2**	○/○/○	○	○	●	●	○	● **P_{ow}**	●	**1/2**
BBP	**85-68-7**	○/○/○	○	○	●	●	○	● **BCF**	●	**1/2**
DIBP	**84-69-5**	○/○/○	**-**	**-**	●	●	**-**	● **BCF**	●	**1/2**
ASE	91082-17-6	○/○/○	-	○	○	●	● (Not readily)	● P_{ow}	○	2/2
ATBC	77-90-7	○/(○)/○	○	○	○	(●)	○	● BCF	●	1/2
COMGHA	736150-63-3	○/○/○	-	○	-	(●)	○	● P_{ow}	●	1/2
DEGD	120-55-8	○/(○)/○	-	○	(●)	●	○	(○) BCF	●	1/2
DINP	68515-48-0 28553-12-0	○/○/○	○	○	(●)	●	○	● BCF	○	1/2
DGD	27138-31-4	○/(○)/○	-	○	(●)	●	○	● P_{ow}	●	1/2
DEHT / DOPT	6422-86-2	○/(○)/○	○	○	○	●	● (inherently)	● P_{ow}	(●)	1/2
DINA	33703-08-1	○/○/○	-	○	-	●	○	(●) (conflicting)	○	1/2
DINCH	166412-78-8	○/(○)/○	○	○	○	●	● (Not readily)	● P_{ow}	○	1/2
GTA	102-76-1	○/○/○	-	○	○	○	○	○	○	1/2
TXIB	6846-50-0	○/(○)/○	-	○	●	●	● (inherently)	○ BCF	●	1/2

Notes:

The inherent properties for the investigated substances are summarised using key parameters: acute and local effects, sensitisation, carcinogenicity(C), genetic toxicity (M), reproductive toxicity (R), persistence, bioaccumulation and aquatic toxicity. If data are not available for all parameters or only from non standard test results a tentative assessment is given (shown in parentheses). The symbols: ● identified potential hazard, ○ no identified potential hazard, and – no data available. () indicate the effects are considered of minor significance.

*1 The terms refer to different biodegradability tests:

Inherently biodegradable: Not meeting the criteria in an "inherent biodegradability" test

Not readily biodegradable: Not meeting the criteria in "ready biodegradability" tests.

*2 ● is based on BCF > 100 or Pow > 3 (BCF prevails over Pow where both values exist).

*3 ●● is used for very toxic and toxic < 10 mg/L.

*4 The following notation is used:

Data quality (first number):

1 Data summaries from recognised, peer reviewed sources (e.g. EU HVP programme, SIDS, SCHENIR, NICNAS) or reliable test data.

2 Data summaries from not peer reviewed sources, considered reliable with restrictions (e.g. IUCLID).

3 Data summaries which do not give sufficient experimental details to evaluate the quality.

Data completeness (second number):

1 Data considered sufficient for classification of CMR effects and according to PBT criteria.

2 Data available about the endpoint, but not considered sufficient for classification.

3 Data not available or relevant for classification of the endpoint.

An average score is assigned based on the sum of scores for C, M, R, P, B and T properties as follows: Sum 6-8=1, Sum 9-14=2 and Sum 14-18=3

As stated above in C.14.1 and C.14.2 it can be concluded that the overall assessment of the alternatives shows, that they do not pose an additional risk compared to the 4 phthalates DEHP, DBP, BBP and DIBP. For some of the endpoints (other than reprotoxicity) some of the alternatives do not seem any better than the 4 phthalates, but since non are classified and the effects of the most used phthalates are less significant compared to the 4 phthalates it can be concluded, that using the alternatives instead of the 4 phthalates in question will result in an overall benefit.

C.15.4 Summary and discussion of technical and economic feasibility of the alternatives

The technical description in this report of eleven selected alternatives to DEHP, DBP, BBP and DIBP is based on the producers' assessments of relevant application fields and experience on the market, as well as the evidence of already established practises, especially in the toys, foodstuffs and medical product fields, but also for other end-uses. Based on the available information, a number of suitable alternative plasticisers have been identified for all applications. Some of the alternative plasticisers have a broad application scope, others are more specialised. A summary of the assessed alternatives is given in Table 106. For some of the substances the manufacturers have provided information on market experience. It has to be mentioned that the information was given in 2009. The data are summarised in Table 107. Some of the substances, for which specific information on market experience has not been supplied (e.g. DINCH), certainly have some experience as indicated by the production volumes of the substances.

So far, the dominance for many years of DEHP and other ortho-phthalate plasticisers may have naturally limited the motivation to get more full scale experience with other plasticiser types. Under other circumstances, driven by other priorities, the experience with so far less favoured plasticisers would inevitably increase. The research would however not have to start from scratch, as many relevant substances have been investigated for plasticiser characteristics in early research and are already used to a great extend.

DINP and DIDP have become dominating alternatives to DEHP due to their closeness in performance to DEHP, their availability and their only moderately higher costs.

In some cases, blends of different alternative plasticisers may be needed to attain the desired technical characteristics. This is also a well known practice with many DEHP uses. More blending may be needed with some of the non-phthalate alternatives to achieve general plasticiser characteristics. By way of example, a Danish toy producing company attempted a switch to ATBC as primary plasticiser in all PVC toys etc. for small children. However in the existing production setup, it suffered from a variety of technical drawbacks when compared with DINP: ATBC would not take decoration, it had high migration into adjacent materials leading to swelling and splitting, and there was a consequent need for tooling changes. Development led to the use of a mixture of ATBC, DINCH and DEHT, which could be blended in a variety of combinations to achieve softened PVC that performed to the required standards with the existing production setup, and could be used as a one-to-one alternative to DINP. Also, some of the marketed plasticiser products consist of several substances, pre-mixed to provide desired performance characteristics. The products Soft-n-safe and Benzoflex 2088 described in this report are examples of such mixed plasticiser products.

Table 106. Summary of the technical assessment of alternative plasticisers

Substance	Overall technical assessment
ASE	ASE is a general plasticiser alternative to DEHP. The producer has indicated significant market experience for most traditional DEHP, DBP and BBP uses.
ATBC	The performance of ATBC on some parameters seems similar to DEHP, indicating technical suitability for substitution of DEHP for some applications. The higher extractability in aqueous solutions and the higher volatility may reduce the performance of ATBC as a plasticiser in PVC. The data available does not allow a closer assessment of ATBC's technical suitability as alternative to DEHP, DBP and BBP
COMGHA	The producer has indicated significant market experience in several of the traditional DBP and BBP specialty plasticiser applications and certain DEHP applications, notably in the non-polymer (adhesives, sealants, etc.) and PVC spread coating (plastisol) application fields. According to the producer, Benzoflex 2088 (with DEGD) has become the main non-phthalate alternative to DBP or BBP in vinyl flooring production in Europe. The higher extractability in water may limit its use for some applications.
DEGD	According to the producer, COMGHA still has relative moderate market experience, albeit with many examples of full scale usage and pilot/lab scale tests, and significant market experience in some plastisol application and cosmetics. The producer found good performance on key technical parameters indicating a potential for substituting for DEHP and perhaps for DBP and BBP in some traditional uses og these substances.
DGD	DEHT is a general plasticiser alternative to DEHP. Today, terephthalates like DEHT are more commonly used in the USA than elsewhere.
DINP	DEHT is a general plasticiser alternative to DEHP and is generally the preferred and most used alternative due to it's low price and the technical suitability.
DEHT	DINA has mostly been used for low temperature PVC applications and in PVC film/wrapping . The data available for this study does not allow clear-cut conclusions as regards DINA's suitability as alternative to DEHP
DINA	The producer's sales appraisal indicates a relatively wide usage of DINCH for general plasticiser purposes. DINCH was the most frequently found plasticiser in two European surveys of plasticisers in toys and childcare articles. The data available does not allow a closer assessment of DINCH's technical suitability as alternative to DEHP, DBP and BBP.
DINCH	The fact that DGD for many years has been a well known and much used competitor to BBP, especially in PVC flooring and in PVA adhesives, indicates a clear potential for substituting DGD for BBP, from a technical point of view. DGD may probably also substitute for some traditional uses of DEHP and DBP.
GTA	According to a producer, GTA can substitute for DBP and BBP in adhesives, inks and coatings. The data available does not allow a closer assessment of GTA's technical suitability as alternative to DEHP, DBP and BBP.
TXIB	TXIB was found in more than 10% of the samples in surveys of plasticisers in toys and childcare articles. However, the producer does not consider TXIB an alternative to DEHP, DBP or BBP, and the usage of TXIB in vinyl flooring has declined in the 1990's due to high emissions from end products. Consequently, TXIB seems not to be a suitable alternative to DEHP, DBP or BBP.

Below in table 107 is given information about possible uses for the available (or proposed by industry) alternatives besides DINP. It has to be underlined once more that DINP is the main alternative used in the vast majority of the applications. It also has to be underlined that it is stated on the DIBP Information Centre's webpage that" *Diisobutyl phthalate (DIBP) has very similar application properties to Di-n-butyl phthalate (DBP) and may therefore be used to substitute for DBP in most, if not all, of its applications. These range from the plasticisation of PVC to the production of paints, printing inks and adhesives."* From this it is obvious that the alternatives to DBP can be used as substitutes for DIBP as well.

Table 107. Alternatives (beside DINP) to DEHP, BBP and DBP proposed by contacted manufactures, by application and with indication of market experience.

Application	ASE	GTA	DGD	Mix of DGD, DEGD, TGD	ATBC	COMGHA
Substitute for DEHP						
Polymer applications:						
Calendering of film, sheet and coated products	2	2	4	4	3	3
Calendering of flooring, roofing, wall covering	4	2	3	3		3
Extrusion of hose and profile	2	2	3	3	3	3
Extrusion of wire and cable	2	2	3	3		3
Extrusion of miscellaneous products	2	2	2	2	2	3
Injection moulding of footwear and miscellaneous	?	2	2	2		3
Spread coating of flooring	2	2	2	2		2
Spread coating	2	2	2	2		3
Car undercoating	2		3	3		
PVC medical articles		2			2	
Toy and childcare articles		2			1	
Non polymer applications:						
Adhesives/sealant, rubber	2	2	1	1	2	4
Lacquers and paint	2	2	2	2		4
Printing ink	2	2	2	2	2	3
Production of ceramics						
Substitute for DBP						
Plasticiser in PVC	2		1	1	2	2
Plasticiser in other polymers	2					2
Adhesives	2	2		1	3	4
Printing inks	2	3			2	3
Miscellaneous:						
Sealants	2				3	4
PU foam sealants	2				4	
Nitrocellulose paints	2	3	2	2	2	
Film coatings	3				3	
Glass fibre production						4
Cosmetics						2
Substitute for BBP						
Polymer applications:						
General PVC (e.g. for moulded plastic parts)	2					4
Plastisol coating, for flooring	2		1	1		3
Extrusion or spread coating	2			2		2
Films, calendering	2		4	4		3
Non polymer applications:						
Sealants	2		1	1		
Coatings and inks)		2	1		3	
Adhesives	2			1		
Nail polish					1	

- *1: Market experience categories interpretation: 1) Main alternative on market. 2) Significant market experience. 3) Examples of full scale experience. 4) Pilot/lab scale experience

C.16 Assessment of alternative flexible polymers

This dossier is in general not addressing other than PVC-based polymers and their alternative plasticers; however in order to give a very short and general description of some of the alternative flexible polymers is below such short background information (taken from Maag et al., 2010).

A number of studies have been undertaken on replacing PVC with other materials for different applications. The conclusion of many studies has been that it - on the basis of the available data - was not possible to make a full assessment of the materials.

The focus is on an assessment of polyolefin (polyethylene/polypropylene) elastomers as alternatives to flexible PVC - on an overall screening level - of its lifecycle impacts, supplemented by brief description of other flexible polymers based on avail-able aggregated reviews. The studies carried out are primarily on medical devices and toys which are not part of the proposed restriction, but the results are believed to be applicable for other articles as well.

Alternative materials for certain medical devices

A detailed life cycle assessment (LCA) was performed by Stripple *et al.* (2007) assessing the life cycle impacts of three flexible polymers in their use as urinary catheters, a disposable medical care product consisting of a thin flexible tube and a cone-shaped flexible connector in one end. The materials assessed were (Stripple *et al.*, 2007, Melitek, 2006, 2009):

- DEHP-plasticised PVC;
- Thermoplastic polyurethane (TPU - The assumed TPU composition studied was based on hydrogenated methylene) diisocyanate (HMDI), polytetramethylene ether glycol (PTMEG) and 1,4-butadiol.
- An elastomer, marketed by Melitek (Denmark) under the product name Meliflex (here designated PO), based on polypropylene, styrene block copolymer polyethylene and (non-phthalate) additives in ppt concentrations.

The functional unit was one year's supply of catheters for one person. The LCA seems comprehensive and was performed by the independent institute IVL in Sweden. It was conducted using 4 different LCA methods, the Ecoindicator 99 system, the CML 2 system, and the EPS 2000 system, as well as a classification and characterisation in line with the EPD system (environmental product declaration system).

The results varied somewhat depending on the assessment methodology used, but in broad lines the TPU elastomer was assessed as having higher environmental impact than the PO elastomer and plasticised PVC, whereas the last two were assessed as having quite equal overall impacts. It should be noted that while some human toxicity aspects were included, the health effects of DEHP could not be included in the assessment, as no conclusive toxicity data suited for the methodology had been identified. The major assessed effects appeared quite influenced by energy resource depletion and energy related emissions.

The overall conclusion can be drawn that a DEHP-free medical grade flexible polymer is available. It is primarily based on low toxic olefins and has similar or lower life cycle impacts than plasticised PVC. The material, Meliflex, is more expensive per weight, but as less material is needed per unit of the medical functions investigated, this partly outbalances the price difference (Melitek, 2009). Meliflex is designed and produced specifically for pharmaceutical packaging and medical devices

applications. Grades are available for tubing extrusion, films blown by cast extrusion, for injection moulding and for blow moulding.

Previously an environmental and health assessment of two alternative materials has been conducted by Stuer Lauridsen et al., 2001: PU (polyurethane) and LDPE (low density polyethylene). On the basis of the available data it was not possible to make a full assessment of the materials. It is however in the report recognised that LDPE has a low toxicity and that LDPE does not release large quantities of monomers or oligomers.

A study on alternatives to soft PVC in building materials among others concludes that polyethylene and other polyolefins have better environmental characteristics than PVC for many applications in the building industry (Andersson, 2002).

As described above, another DEHP-free flexible medical grade material, TPU, is available. The LCA performed by Stripple *et al.* (2007) indicated, that its life cycle is more energy-intensive and have higher emissions of prioritised pollutants. According to Stripple *et al.* (2007), while plasticised PVC is the traditional flexible material of choice in the medical market, TPU also has a significant part of the market. No price data have been collected for TPU for this study.

Alternative materials for toys

Postle et al. (2000) note that a number of companies have undertaken substitution to entirely different plastic products rather than using different plasticisers. For those products which are specifically intended to be placed in the mouth, the substitute plastics which appeared to be most widely used were polyethylene (PE) and ethylene vinyl acetate (EVA). These materials can reportedly be used adequately in the products in question. However, the technical performance of the final product has been indicated to be often slightly inferior to that obtained with PVC. For example, products produced from these materials may sometimes have lower resistance to biting and tearing than plasticised PVC. The products may also have reduced longevity. In terms of the wider range of toys and childcare articles, plastics which are reported to be used as substitutes for plasticised PVC include various forms of polyethylene (LDPE, and LLDPE) styrenic block copolymers and again EVA, as shown below.

Summary of technical suitability and use of alternative flexible materials for use in toys (Postle, et al, 2000)

Table 2: Technical Suitability of Substitute Plastics		
Plastic Type	**Technical Suitability**	**Actual Use as Substitute**
Polyethylene (various forms)	I, II (some)	I, II (some)
Ethylene Vinyl Acetate (EVA)	I, II (some)	I, II (some)
SBS Block Copolymers	I (possibly), II (some)	I (unknown), II (some)
Polyester Elastomers	II (some)	Unknown
Key: I - products intended to be placed in the mouth II - other toys and childcare articles		

Review of life cycle assessments (LCA) of PVC and alternative materials

In a study for the European Commission, Baitz *et al.* (2004 compiled an overview of the publicly available information on LCA on PVC and competing materials, for a variety of applications. Approximately 100 LCAs related to PVC were identified, of these 30 included comparisons at the

application level. For roofing applications the study concludes that higher quality of the systems (thermal conductivity per thickness of roofing sheet layers) as well as the accuracy of the laying and maintenance processes have a large influence over the reduction of environmental impacts. Additionally, the study concludes that 'green roofing' (e.g. planting on the roof) further decreases environmental impacts because of the subsequent longer lifetime of the roofing systems. Three polymer solutions (one PVC system and two competing systems) have the potential to perform better, with similar environmental impacts on global warming, acidification and ozone formation over the life cycle. The study reports that some polymer solutions tend to have lower environmental impacts than competitive systems. Few comparative LCA studies pertaining to consumer goods are available. No useful general conclusions on material comparisons could be drawn.

In a review of various studies on alternative materials to plasticised PVC it is concluded that the available reviewed studies demonstrate that for many applications of DEHP/PVC alternative materials exist at similar prices (COWI, 2009a). Many of the materials seems to have equal or better environmental, health and safety, performance and cost profiles, but clear conclusion are complicated by the fact that not all aspects of the materials' lifecycles have been included in the assessments.

Pedersen (1999) produced a simplified matrix indicating a gross differentiation of polymer materials in some overall categories of health and environment impacts. It is presented in the table below that is based on its presentation in the publication Nordic Ecolabelling (2007). Being simplified, and being based on late 1990 knowledge, the table should likely be interpreted with some caution. Note that the low impact classes 1 and 2 include the substances polyethylene (PE), poly(isobutylene) (PIB), ethylene vinyl acetate (EVA), styrene ethylene butylene styrene co-block polymer (SEBS), styrene isoprene block polymer and silicone.

Conclusions

A number of flexible polymers are available which can substitute for many traditional uses of flexible PVC. Polyethylene (PE), polyolefin elastomers, different polyurethane (PU) qualities, ethylene vinyl acetate (EVA) and different rubber types are examples of among others. For many flexible PVC uses, also other substitute materials than flexible polymers exist. The LCA-based, application-focused assessments are few, and often clear-cut conclusions could not be made. But many materials exist with seemingly equal or better environmental, health and safety, performance and cost profiles. The assessment made here does not allow for a more detailed analysis of possibilities and limitations in the coverage, including financial aspects, of alternative flexible polymers.

Simplified categorisation of polymers according to overall health and environment pressure (From Pedersen, 1999, as cited by Nordic Ecolabelling, 2007; extracts on flexible polymers)

Category	Description	Material
1	The polymer materials in this category contain particularly health or environmentally hazardous substances, which are crucial for the manufacturing or for the properties in use of the polymer. The substances added or generated in the production, use or disposal phase may require special end-of-pipe precautions or protective equipment and may result in significant health or environmental impacts. It should be noted that where the necessary end-of-pipe precautions and protective equipment are adequately installed during manufacturing, the impacts on health and environment can be made negligible.	Polyethylene – PE Poly (isobutylene) – PIB Ethylene vinyl acetate – EVA
2	The polymer materials in this category contain health or environmental hazardous substances, which are crucial for the manufacturing or for the properties in use of the polymer.	Styrene ethylene butylene styrene co-block polymer – SEBS

	The substances added or generated in the production, use or disposal phases may not according to law, require any special end-of pipe treatment or special for protective equipment but might have health or environmental impacts. The polymer materials, which fulfil the first criteria in Category 1 but require large energy consumption to manufacture or which generate relatively low levels of energy upon incineration, are also listed in Category 2.	Styren isoprene block polymer Silicone
3	The polymer materials in this category contain particularly health or environmentally hazardous substances, which are crucial for the manufacturing or for the properties in use of the polymer. The substances added or generated in the production, use or disposal phase may require special end-of-pipe precautions or protective equipment and may result in significant health or environmental impacts. It should be noted that where the necessary end-of-pipe precautions and protective equipment are adequately installed during manufacturing, the impacts on health and environment can be made negligible.	Latex/Natural rubber (cispolyisoprene) – NR Polyvinyl chloride not plasticized with DEHP – PVC (soft) Thermoplastic Polyurethane – TPU Polyurethane foam – PUR foam
4	The polymer materials in this category are regarded as particularly hazardous to health and environment. This category includes polymer materials that otherwise would be in category 1-3 but which contain additives considered as hazardous to health and environment.	Polyvinyl chloride plasticized with DEHP – PVC(soft) Halogenated additives Additives with heavy metals Fire-retardant based on bisphenols or diphenyl Plasticizers based on DEHP Other additives with the ability to act as endocrine disrupters

C.14.4.1 Prices of alternative plasticisers

Some of the alternative plasticisers investigated have similar or only slightly higher prices than the relevant competing substances among DEHP, DBP and BBP; see Table 106 below. Others have higher or substantially higher prices. As shown in the table, the ortho-phthalate alternatives DINP and DIDP as well as the non-phthalate alternatives DGD, DEGD and DEHT were in the same price range as the DEHP, DBP and BBP,), whereas ASE, DINA , DINCH and GTA were somewhat more expensive and ATBC and COMGHA were considerably more expensive (counted as direct relative price). It has not been possible to find a price for TXIB. Note that here BBP and DEHP had the same price per weight unit in this case. In older literature, BBP is reported to be a specialty plasticiser with higher price than DEHP.

Prices of chemicals (and other industrial products) tend to decrease as production capacity and competition is increased. Different chemicals are however based on different raw materials and more or less complex and resource demanding chemical synthesis technologies. This of course sets limits to the minimum prices attainable even in a mature market, and some of the alternative plasticisers described may likely remain at higher price levels. It should be noted that the prices of DEHP have dropped significantly over the last decade or more.

Under the conservative assumption that production prices of plasticised PVC on average would rise corresponding to a 11% raise in the plasticiser price in a competitive market, the increased cost of substituting 1 tonnes of DEHP by DINP would be approximately 100€/ton.

Table 106. Prices and relative prices to DEHP of the assessed alternatives and some other reference plasticisers

Substance	Price	Relative price to DEHP,% *2	Substitution factor *2	Effective relative price,% *2	Remarks
Phthalates and other reference plasticisers:					
DEHP (2006)	0.70 USD/Lb	-	-	-	TURI (2006)
DEHP (2006-2009)	≈0.8-1 €/kg	-	-	-	ExxonMobil (2009), Arbeitsgemeinschaft PVC (2006)
BBP (2006)	0.70 USD/Lb	100%	0.94	94%	TURI (2006)
DINP (2006)	0.73 USD/Lb	104%	1.06	111%	TURI (2006)
DIDP (2006)	0.77 USD/Lb	110%	1.10	121%	TURI (2006)
Assessed alternatives:					
ASE (2009)	1,75 €/kg	175% *1	NA	NA	Lanxess (2009)
ATBC	NA	300%	NA	NA	ExxonMobil (2009); Karbæk (2003)
COMGHA	3.5€/kg	≈350% *1	≈1	≈350%	Danisco (2009)
Benzoflex 2088 (with DEGD)		"Slightly higher"			Genovique (2009)
DGD (2006)	0.73 USD/Lb	104%	0.98	102%	TURI (2006)
DEHT (2006)	0.74 USD/Lb	106%	1.03	109%	TURI (2006)
DINA		150-200%	0.98	150-200%	ExxonMobil (2009)
DINCH (2006)	0.91 USD/Lb	130%	NA	NA	TURI (2006)
GTA	€1,50/KG	150% *1	NA	NA	Lanxess (2009)
TXIB	NA	NA	NA	NA	

Notes: *1: DEHP price in 2006-2009 ≈0.8-1 €/kg; 1 € is used for calculations. NA = Not available for this study.

*2: "Relative price to DEHP" indicates the price of the alternative plasticiser pr weight unit in relation to the price of DEHP. The "substitution factor" indicates how much of the alternative plasticiser that is needed for replacing DEHP in order to have the same plasticising effect. The "Effective relative price" is a combination of the two measuring the cost of substituting DEHP with the alternative plasticiser in order to gain the same plasticising effect in the final material.

D. Justification for action on a Community-wide basis

D.1 Considerations related to human health and environmental risks

As explained in section B, the hazard properties of the four phthalates are widely recognized as they are classified as Repr. Cat 1B.

As reported in Section B.2.2, the four phthalates are used in a variety of consumer articles throughout the EU. Consequently, emissions originating from the articles during their indoor use, take place in all the Member States, even though the emissions in different parts of the EU vary depending on the status of the switch to articles with alternatives to the four phthalates due to awareness of both consumers and producers/importers.

Therefore, the risks need to be controlled on a Community-wide basis.

D.2 Considerations related to internal market

The proposed restrictions cover articles that are extensively traded among and used in all Member States.

Articles containing one or more of the four phthalates are produced in as well as imported into the EU. Especially PVC has a very widespread use in all Member States both for consumer products as well as for industrial uses. The justification to act on a Community-wide basis origins from the need to avoid different legislations in the Member States with the risk of creating unequal market conditions. The proposed regulation of the 4 substances through Community-wide action ensures a 'level playing field' among all producers and importers of the devices throughout the Community.

D.3 Summary

The main reasons to act on a Community-wide basis is the cross-boundary human health problem, the severity of the possible health risk as documented in section B of this dossier, and the extent of the risk (most children and adults are in daily contact with some of the articles or are staying in indoor air exposing them to the four phthalates). Furthermore, the fact that the articles containing the four phthalates, imported as well as produced in EU, need to be restricted on a common basis within the EU stresses the importance of the Community-wide action in order to avoid market distortion. In this respect the Authorisation process under REACH only covers use within the EU and thus not imported articles and this would distort competition on the European Market. Thus, the use of the four phthalates in these articles needs to be controlled at the EU level.

E. Justification why the proposed restriction is the most appropriate Community-wide measure

This part provides the justifications why the proposed restriction is the most appropriate Community-wide measure. It gives an overview of the assessment of the proposed restriction against effectiveness, practicality and monitorability of the restriction including assessment of other risk management options. It also includes an assessment of the current and future use of the four phthalates and their alternatives.

E.1.1 Risk to be addressed – the baseline

Trends for further substitution

As shown in Section B.2.2 alternatives to the four phthalates are available on the market for all fields of application considered. The alternatives available may include other plasticisers as well as other types of materials. No examples have been identified of products for which alternatives are not available.

The dominant alternatives to the four phthalates reported for the uses in question are DINP and DIDP. For most applications, however, non-orthophthalate plasticisers such as DOPT, and other plasticiser types such as DINCH, ASE, ATBC, COMGHA and dibenzoates are actually in use, or being considered by producers as realistic alternatives to ortho-phthalates.

According to Table 7 articles placed on the market for use indoors and/or direct human skin contact in total in 2007 is estimated to contain 265,000 tonnes of the four phthalates, DEHP, BBP, DBP and DIBP. DEHP represents 95 percent of this. The imported articles represented at least 40,000 tonnes (16 percent).

Based on information in registrations submitted by producers and importers the content of the four phthalates in articles seems to be have been reduced by 36 % from 2007 to 2009-10 to 170,000 tonnes. (cf. B.2.1).

The information collected shows that the four phthalates are still used in large quantities in articles for use indoors and/or with direct human contact. But for many fields of application voluntary phasing out of the four phthalates and/or phthalates in general has already taken place, or is in the process. The substitution process has been in process during the last 10 years and has apparently accelerated after the inclusion of the three phthalates into Annex XIV to REACH (see below).

Important milestones for the phthalates covered by the proposed restriction are:

- DBP and DEHP classified as repro toxic: 2001
- BBP classified as repro toxic: 2004
- DEHP, DBP and BBP Restricted in Toys and childcare products: 2005
- DEHP, DBP and BBP included in Candidate List: October 2008
- DIBP classified as repro toxic: 2009
- ECHA recommend DIBP to the Candidate List: 2010
- DEHP, DBP and BBP included in Annex XIV: 17 February 2011
- Latest day for application for authorisation: 21 July 2013 (DEHP, DBP and BBP)
- Sunset date: 21 February 2015 (DEHP, DBP and BBP)

Substitution to other plasticisers than the four phthalates is expected to continue at least for uses where the costs are considered to be limited[2]. This will be supported by the requirement in REACH of the supplier of an article containing one or more of the four phthalates in a concentration of more than 0.1 % to inform the customer (upon request) if the article contains one or more of the four phthalates. It is therefore assumed that the amount of articles containing the four phthalates will be reduced.

[2] No information has identified areas where the cost of substitution is high, cf section C.

Defining a baseline

Three of the four phthalates (DEHP, DBB and DBP) have been included in Annex XIV to the REACH Regulation. Therefore after the sunset date of 21 February 2015 it will only be possible to use these substances in the production of articles if an authorisation for the specific use has been granted and only for articles.

This has a consequence for setting up "a business as usual" scenario. One could argue that the inclusion in annex XIV of the three phthalates implies that almost all EU use of all four phthalates will be phased out by 2015, assuming that no or very few authorizations will be granted[3]. The benefits and costs related to this proposal for a restriction would then be limited to health benefit and incremental cost for avoiding imported articles.

However, according to Art. 60(6) in REACH it is not possible to apply for an authorisation to use an Annex XIV substance if this would constitute a relaxation of a restriction set out in Annex XVII. This implies that a restriction will have direct consequence for the possibilitity to apply for an authorisation. One could therefore also argue that the baseline scenario should be more hypothetical assuming that the inclusion of the substances in Annex XIV is not taken into account when setting up the base line. This would make it possible to evaluate the impacts of a ban, whether this is due to the proposed restriction or to a rejection of an authorisation application. The only element related to the authorisation procedure to be included in the base line would be dynamic effects following industry considerations on the possibility to substitute the four phthalates, which have to be made whether or not industry would apply for an authorisation.

Business as usual – without taking the inclusion of substances into Annex XIV into consideration.

Taking into account the dynamic effects mentioned in the previous paragraph a hypothetical baseline scenario could assume that the content of the four phthalates in articles for use indoors /direct skin contact in EU produced articles will decrease by 5% per year and that the substitution in imported products will decrease by 3% per year in the period from 2010 to 2015. The total content of the four phthalates in 2015 will thus be 135,000 tons, of which 30,000 tons will be in imported articles. From 2015 the substitution rate in relation to the four phthalates is assumed to be 3% for all articles, implying that the content of the four phthalates in the mentioned articles in 2020 will be 115,000 tons of which 26,000 tons will be imported. It is important to stress that these figures are given with great uncertainty.

Businesses as usual - taking annex XIV regulation into consideration.

Considering only phthalates in articles not produced within the EU (as those produced within EU are covered by the authorisation scheme also expected inclusion of DIBP in Annex XIV) the baseline would be the imported articles – which imply an aggregated content of phthalates in imported articles of 40,000 tonnes in 2007, 35,000 tonnes in 2009-2010, 30,000 tonnes in 2015 and 26,000 tonnes in 2020.

[3] it is not possible to grant authorisations for uses that would constitute a relaxation of a restriction (REACH regulation Art 60(6) and it is expected that this proposal for a restriction will enter into force before 1 February 2015 – the sunset date in the authorisation scheme.

E.1.2 Options for restrictions

The relevant risk management option (RMO) should address exposures caused by emissions from articles that emit the phthalates DEHP, DBP, BBP and DIBP to the indoor environment as well as from articles where there is a direct exposure through contact to humans. Especially exposure of children is in focus.

The outset is that the exposure to the four phthalates from dust and food and all additional exposure will result in a RCR that will exceed 1, which means that there is a risk for effects.

Four options are explored (see section E.2):

RMO 1: (the proposed restriction) – restriction on articles for use indoors and human skin contact

The proposed restriction will ban the placing on the market of all articles intended for use indoors or articles that may come in contact with the skin or mucous membranes if the articles contain one or more of the four phthalates DEHP, DBP, BBP and DIBP in concentrations above 0.1 % of any plasticised parts.

RMO 2: Wider scope: - restriction on all articles

In this restriction placing on the market of all articles containing one or more of the four phthalates in a concentration above 0.1 % is banned.

RMO 3: Narrower scope – restriction on identified groups of articles

In this restriction placing only includes articles in specifically mentioned groups of articles. Placing on the market of such articles containing one or more of the four phthalates in a concentration above 0.1 % is banned.

RMO 4: Migration based restriction

In this restriction the restriction is based on migration of the four phthalates in stead of content. In fact this RMO can be considered as a variation of the RMO1, RMO2 and RMO3.

E.1.3 Other Community-wide risk management options than restriction

Possible Community-wide risk management measures other than a restriction are outlined in table 107 below. However, it is concluded that none of these are realistic, effective and proportionate means of solving the problem. As such, none of these other risk management options have been considered further within this analysis.

Table 107. Possible other Community-wide options discarded at this stage

Option	Reasons for discarding this option
EU-legislation other than REACH	
Control of emissions under the IED and/or Water Framework Directive and waste legislation	Articles containing the four phthalates have wide dispersive use. For the general public exposure caused by emissions to indoor environment or direct contact occurs during the use phase, not the production phase. Measures aimed at point sources would not solve the problem.

Option	Reasons for discarding this option
Sector specific legislation	Uses are varied and widely dispersed – it would be very difficult to apply and enforce to a large number of subsectors.
Voluntary industry agreement	There is no specific industry sector to make an agreement with. There are several thousands of both small, medium sized and big importers of articles that could contain the four phthalates and there are also a lot of producers of articles containing the phthalates that are not organized in the main European associations. This lack of organization and the number of importers and producers will also affect the possibility to monitor effectiveness of the desired effect of the proposed measure. An organisation, Vinyl 2010, representing the whole (organized) PVC industry chain has been set up to provide the organisational and financial infrastructure to manage and monitor the implementation of the Voluntary Commitment of the European PVC Industry. This is a 10-year plan to put the PVC industry at the forefront of sustainability by minimising the environmental impact of the PVC production, promoting responsible use of additives, supporting collection and recycling schemes, and encouraging social dialogue between all of the industry's stakeholders. However this organisation does not represent importers of articles
Information to consumers and retailers incl. labelling	To retailers – Avoid selling the articles in question. This RMO does not seem to be sufficiently effective. Although the classification of the substances as Rep Cat 2 according to Dir. 67/548/EEC has been in force for many years, the articles still contain the substances. To consumers – Avoid buying the articles in question. This RMO does not seem to be sufficiently effective. For the consumer it will be difficult to identify the articles containing the substances. Even if the articles are labelled it is a problem that some of the articles have a long life expectancy, e.g. PVC flooring. A house might change owners/tenants within the lifespan of PVC flooring. Therefore the person that is exposed might be another than the one taking the buying decision. To consumers – Ensure sufficient ventilation. Calculations show that this will not be effective as the concentration in indoor air still will be too high when the exposures from other sources are taken in to account. Besides, it has to be remembered that the articles will continue to emit the phthalates throughout their lifetime and absorp to other articles and surfaces. This RMO will also not cover direct exposure from articles from e.g. skin contact. However this RMO would address some of the risks from articles already in use. The RMO can be supplementary to a restriction in relation to existing articles. Such advice can be given on national orlocal level
ROHS	Electrical and electronic products are regulated by the RoHS Directive, and DEHP, BBP and DBP are subject to - as part of the first-coming review - to be addressed in order to investigate the necessity to include these substances in the Directive. Such a review is restricted to the use in electric and electronic devices and does not consider other articles and the combined exposure from all articles.
Product safety Directive 2001/95/EC	Does only address risks related to specific articles and not risks related to a cumulated exposure from different articles
Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food	Regulation no 1935/2004 only addresses risks from materials and articles intended to come into contact with food, and does therefore not address the risks from the articles addressed in this dossier. According to Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food DIBP may not be used, while as technical support agents DEHP and BBP are allowed in concentrations up to 0,1 % in the final product, DBP is allowed in concentrations up to 0,05 % in the final product
Other REACH processes	

Option	Reasons for discarding this option
REACH Authorisation process	3 of the phthalates (DEHP, DBP and BBP) have been included in the authorisation list (Annex XIV). The authorisation route only addresses use within the EU. This means that risks related to the placing on the market of articles are not addressed. The timeframe for the proposal will probably also be much shorter than what can be achieved via the authorisation route. Furthermore the authorisation route would look upon the individual substances and in individual articles while this proposal deals with the combined effects of the phthalates from a broad range of articles. The 4th phthalate (DIBP) is on the candidate list – and the same reasoning as given for the above mentioned 3 phthalates are adequate here recognising that the process is not as advanced as for the 3 others). DIBP is listed on ECHA's 2nd recommendation for inclusion into Annex XIV. Another point is that the authorization process will take a long time to implement. The sunset date is 21.02.2015. See also section E.1.1.
REACH Art. 68.2	REACH Article 68.2 stipulates that substances that are CMR categories 1 or 2 can be subject to a proposal from the Commission to inclusion in Annex XVII without using the procedures in article 69-73 in the REACH Regulation. The character of this proposal is however such that the procedures in article 69-73 are of major importance for the processing of a dossier this complicated. Discussions and opinions from independent experts are essential due to the fact that this is the first time that the combined and additive effects are used as reasoning for proposed restriction.

E. 2 Assessment of risk management options

E.2.1 Restriction management option 1 (the proposed restriction)

The proposed restriction will ban the placing on the market of all articles intended for use indoors or articles that may come in contact with the skin or mucous membranes if the articles contain one or more of the four phthalates DEHP, DBP, BBP and DIBP in concentrations above 0.1 % of any plasticised parts. See discussion of the scope in section A.1.2.

E.2.1.1 Assessment of effectiveness

E.2.1.1.1. Risk reduction capacity

The overall objective of the proposed restriction is to avoid emissions and exposures causing negative impacts on human health from the combined exposure to the four phthalates from articles. The four phthalates are all classified based on their reproductive toxicity (Repr. Cat. 1B, according to the CLP Regulation). The risk characterisation is based on exposure from food, indoor environment and direct exposure from articles. Furthermore the risk characterisation considers similar mode of action of the phthalates which implies that the exposure from the various phthalates are added taking the relative potency of the phthalates into consideration.

The proposed restriction will result in a significantly decreased exposure of the public to the four phthalates. It will not be a complete elimination as the exposure from dust will continue as long as there are products emitting phthalates and there could still be exposure from food as the phthalates may still be found in the environment. On the long term the exposure from these sources will also

decline as a result of the proposed restriction as the articles that are proposed banned contribute to the general load of the phthalates as such.

The most likely alternatives to be used are DINP and DIDP. All studies showing that DINP has anti-androgenic effects on reproductive development show that DINP is much less potent than DEHP, DBP, DIBP and BBP. According to table 15 NOAEL (LOAEL) for DBP) for the four phthalates is between 2 and 125 mg/kg bw/day, while the level of NOAEL for DINP for antiandrogenic effects is 300 mg/kg bw/day. The NOAEL for the most used of the three phthalates DEHP is 4.8. For DIDP there are no indications of anti-androgenic effects.

E.2.1.1.2. Proportionality

Technical feasability

As described in C.14.4 a number of suitable alternative plasticisers have been identified for all applications. Some of the alternative plasticisers have a broad application scope, others are more specialised. For some of the substances the manufacturers have provided information on market experience, summarised in table 105.

DINP and DIDP have become dominating alternatives to DEHP due to their closeness in performance to DEHP, their availability and their only moderately higher costs (see below).

In some cases, blends of different alternative plasticisers may be needed to attain the desired technical characteristics. This is also a well known practice with many DEHP uses. More blending may be needed with some of the non-phthalate alternatives to achieve the desired plasticiser characteristics.

Cost of substitution

Substitution of the classified phthalates with DINP or DIDP generally seems to be the least expensive option, as these phthalates for most applications can substitute directly for the four phthalates with no major changes needed in the production process. The effective relative price of DINP (reflecting both the price per kg and the need for more plasticiser to obtain the same effect) was in 2006 about 11% higher than the price of DEHP (TURI 2006), see table 106. The relative price of DIDP was about 21% higher. As the concentration of plasticisers in the polymer matrix can be up to 40% of the product by weight, the price of the alternatives will influence the price of such final products. However, the cost of plasticisers comprises only a minor part of the total production price of the article (see examples below).

A rough estimate of the increase in total annually raw material cost in order to substitute the four phthalates is based on the assumed content of the four phthalates in 2015 at 134,300 ton (baseline scenario) and a price increase for substitutes in the range of 11 – 21 %[4] compared to the price of DEHP. This would result in total extra cost of € 15 – 30 millions per annum (DEHP price at 1 €/kg).[5] Since the cheapest alternative plasticiser, DINP, will be a possible substitute in most applications the extra cost would be closer to € 15 millions.

In 2015 imported articles are estimated to contain 30,000 tonnes of the four phthalates implying that the extra cost in imported product in total will be € 3 – 6 million.

[4] Table 108

[5] This also includes the use in exempted areas, e.g. in medical devices. However this is not expected to be a high volume

Consequences for certain types of articles

Vinyls for flooring and wall covering

Based on the import and production statistics provided in (Danish EPA, 2011), and the plasticiser concentrations between 10% and 30%, the total plasticiser consumption for EU produced flooring is expected to be within the range of 140,000 - 430,000 tonnes per year. According to manufacturers, most of this is presumably DINP. Similarly, the content of plasticisers in imports is expected to be in the range of 11,000 - 34,000 tonnes per year. In imports, the frequency of DEHP usage may be larger, as DEHP is still the dominant general plasticiser globally (50% of total phthalates consumption in 2007 across all applications according to ECPI [ECPI, 2010].

The content of the four phthalates in flooring vinyls[6] was estimated to about 44,600 tonnes in 2007 (table 7). Assuming a price of the four phthalates of 1 € per kg the price and - as the main substitution plasticiser is considered to be DINP - an 11 % rise in cost due to substitution, the additional cost would be € 4.9 million. Taking the overall estimated reduction[7] of the content of the four phthalates into consideration the remaining content of the four phthalates in 2015 would be 23,000 tonnes[8] additional cost in 2015 is € 2.5 million.

Danish EPA (2011) has estimated the total production value (EU-production + import) to € 2.3 billion. Assuming that the exported articles contain the same concentration of the four phthalates as imported and EU produced flooring and assuming the price for manufactured, imported and exported flooring articles are the same, the total value of flooring articles on the EU market is € 2.0 billion. Thus the additional cost is 0.1-0.2% of the market value of the flooring articles.

The extra costs can also be compared with the value of the flooring articles still containing the four phthalates, namely € 280 million. This is calculated assuming that DEHP accounts for 14% of the plasticisers in flooring products[9]. and assuming similar prices for flooring articles with DEHP and other plasticisers. The additional cost for the producers that have not already substituted the four phthalates is € 3.8 million, which is 1.3% of the total value (2007 values). This calculation does not include wall covering.

The content of DEHP in imported flooring articles are 2,000 tons (table 7), with an estimated value of € 2 million implying additional cost of € 200,000 when substituting to DINP. Taking the overall estimated reduction (30,000/40,000 = 0.75) of the content of the four phthalates in imported products into consideration the additional cost in 2015 is € 150,000.

Cables and wires

Based on import and production statistics plus information from cable manufactures Danish EPA (2010) has estimated the total plasticiser consumption for EU-produced cables to be within the range of 190,000 – 550,000 tonnes per year. The majority of this (about 80 %) may be DIDP, similarly, the consumption in imports is expected to be in the range of 30,000 – 100,000 tonnes per year, DEHP assumed to be more frequently used.

[6] Including 'wall or ceiling coverings of plastics' applies to products in rolls, of a width not less than 45 cm, suitable for wall or ceiling decoration, consisting of plastics fixed permanently on a backing of any material other than paper, the layer of plastics (on the face side) being grained, embossed, coloured, design-printed or otherwise decorated.
[7] From 254,900 tonnes to 121,793 = reduction of app. 50% of EU produced and 25% of imported products.
[8] (37.3+11-4.8-1,6)*0.5+(2+0.7)*75=23,000 – cf. table 7
[9] Based on Danish EPA (2010, 2.1.3), assuming an average plasticiser concentration of 20%

According to table 7 the content of DEHP in cables and wires for indoor use is estimated to be 52,600 tonnes in 2007[10]. With a 21 % increase in cost (Danish EPA, 2010), the extra cost when substituting to DIDP in cables and wires for indoor use is estimated to € 11 million. Taking the overall estimated reduction of 50 % of the content of the four phthalates of EU produced articles and a reduction of 25 % of the content in imported articles into consideration in 2015 the content in the remaining cables is 30,000 tonnes and the additional cost is 6.4 million €.

The total production value (EU-production + import) is about 19.3 billions €[11] (Danish EPA 2010). Assuming that the price for manufactured, imported and exported cables and wires are the same the total value of cables and wires on the EU market is 17.4 billion €. Thus the additional cost is approximately 0.05% of the market value for cables and wires (2007 values).

Assuming that DEHP accounts for 12.4% of the plasticisers in cables and wires[12] the extra costs can also be compared with the value of the cables and wires still containing the four phthalates, namely € 2.15 billion. The additional costs for the producers that have not yet substituted the four phthalates is € 11 million,which is 0.5% of the total value. (2007 values)

The content of DEHP in cables and wires are 6,200 tons (table 7), with an estimated value of € 6.2 million implying additional cost of € 1.320,000 when substituting to DINP. Taking the overall estimated reduction (30,000/40,000 = 0.75) of the content of the four phthalates in imported products into consideration the additional cost in 2015 is € 1 million.

Film/sheet, coated and moulded products

According to table 7 the content of the four phthalates in coated fabric and other plasticiser is 97,400 DEHP tonnes and 110 tonnes BBP. Additional cost of substituting these substances with DINP is about € 10.7 million, using the effective relative prices as given in table 106. Taking the overall estimated reduction of 50 % of the content of the four phthalates of EU produced articles and a reduction of 25 % of the content in imported articles into consideration in 2015 the content in the remaining cables is 53,600 tonnes[13] and the additional cost is € 5.9 million.

There is no data on the total value of the final articles in the group, so it is not possible to relate the costs of substitution to the value of articles.

Table cloth, gloves, imitation leather

Some of articles might contain up to 90 % PVC. Assuming a content of phthalates to be 30%, taking into account that the relevant substitute is DINP (effective price 11% higher than DEHP), a DEHP price on 1 € per kg and using the average price per article for tablecloths, curtains, shower curtains and similar items (not industrial uses) of € 3.3 pr kg[14] the extra cost accounts for 1%[15] of the value of the articles.

[10] This is app. 82 % of the content in all cables and wires, both for indoor and outdoor use. The real proportion of cables for indoor use is smaller as cables included in table 7 are all cables not above 1000 kW, while some cables below that capacity are for use out doors.
[11] Calculated on the basis of Danish EPA 2011 , table 2.2.1
[12] Based on (Danish EPA, 2011, table 2.2.3), assuming an average plasticiser concentration of 25% and an average weight of insulation of 50% of the total cable/wire the 52,600 tonnes DEHP equals 420,000 tonnes cables and wires to be compared with total tonnage of EU production and import of 3.4 million tonnes.
[13] (97.5-18.5)*0.5+18.5*.75= 53,600
[14] Calculated on basis of Danish EPA 2011 (table 2.4.1)
[15] (0.9*0.3*0.1)/3

Bathing and swimming equipment, film for packaging

Danish EPA (2010) has estimated the content in imported bathing equipment (swim-coats, wings, belts and pools) to 1,500 – 1,800 tonnes of DEHP per year.

30 % of the products are made of 100% PVC. Assuming the content of phthalates to be 30%, taking into account that the relevant substitute is DINP (effective price 11% higher than DEHP), a DEHP price on 1 €/kg and using the average price per article for tablecloths, curtains, shower curtains and similar items (not industrial uses) of 3.1 €/ kg[16], the extra cost accounts for 1%[17] of the value of the articles. The substitution cost in 2007 is estimated at € 181,000.

Similar conclusions can be made on plastic sacks, where the average price per kg is calculated to 3.6 €/kg[18]. For more expensive articles the rise of cost is relatively smaller.

Electronic products (EEE)

For most EEE, the flexible parts which may contain the four phthalates comprise only a minor fraction of the equipment and represent only a minor part of the total production price of the product. With a total content of 5,000 - 20,000 tonnes DEHP, the total extra material costs would be 0.5-2 million € per year. No estimates of DBP consumption in EEE have been found. In a survey of their members European Plastic Converters (EuPC), has not indentified any use of DBP, and assume that DBP today is used by relatively few companies for different niche purposes. No estimates of BBP consumption in EEE have been found. An estimated 8,000 tonnes of BBP is used annually for production in the EU, a minor share of this consumption may be used in EEE parts, probably well below 10% considering the many other potential application areas. The consumption of BBP in EEE produced in the EU is therefore likely in the range of 20-200 t/y (Danish EPA, 2010d).

Increases in consumer prices for the individual EEE as a result of a restriction for use in EEE are therefore expected to be small or even negligible. A restriction may impact a large share of all EEE and thereby have an impact on costs for compliance control (Danish EPA, 2010d).

Proportionality between restricting different groups of articles

As mentioned in C.14.4.1 the extra cost in relation to substitution to the two most likely substitutes, DINP and DIDP, are 11 and 21 procent. The cost related to the relatively more expensive DIDP can be compared to the fraction of the phthalates that are emissioned during the articles life time in order to express the cost per reduced emission of the phthalates to indoor air. This is considered to be a relevant parameter for evaluating the cost effectiveness of restricting a group of articles for indoor use. In table 108 lifetime emission factors for several products are compared with extra costs when substituting to alternatives.

[16] Calculated on basis of Danish EPA 2011 (table 2.4.1)
[17] (1*0.3*0.1)/3
[18] Based on Danish EPA 2011 table 2.3.1.

Table 108. Cost effectiveness of substituting the four phthalates in different groups of article

	Tonnage of the DEHP, BBP and DBP in 2007 1000 t	Typical content of phthalates % (average) B	Life-time emis-sion factor % of content of new products c	Alternative d	Extra cost using alternatives e	Cost/effectiveness = c*10/e F
Flooring	34.6	10-20 (15)	0.04	DINP	11%	3.6
Wall covering	10.1	30	0.03	DINP	11%	2.7
Cables and wires, average	52.6	22.5	0.15	DIDP	21%	7
Film/sheet	44.0	30	0.32	DINP	11%	29
Coated fabric	31.8	30	0.05	DINP	11%	4.5

Source: (ECHA, 2009c, Table 2-10) and this dossier table 106.
Note: The life time emission factor is dependant on the vapour pressure of the substance and the difference between the values reflects differences in thickness of the material. The value given in the table is for DEHP, which has a different from DINP and DIDP. However the values for damp pressure for DINP and DIDP are close, and therefore it is relevant to use the value when comparing the two.

From table 108 it can be seen that due to the fact that the emission factor for cables and wires are four times higher than the emission factor for flooring products it is more cost effective to restrict this group of articles even if the price per kg plasticiser used in the article is higher.

As the article group “film/sheet” is the type with the thinnest material the cost-effectiveness for that article type is obvious the highest.

However the cost/effectiveness ratio is in the same order of magnitude and therefore it is not justified to exclude the least cost/effective product group from the restriction.

For products where the risk is related to dermal exposure, it is not possible to establish a similar comparison of the relative contribution from individual types of articles.

Another relevant parameter is whether some groups of articles are expected to imply a total high emission of phthalates due to the total volume of plasticised materials indoors, implying a relatively high concentration of phthalates in indoor air where the products are used. This might be the case for flooring, wall and other articles like waterbeds etc. So even if the ratio per kg might be smaller for these groups the total emission indoor where the articles are used might be high.

It can be concluded that there is no reason to justify an exemption of specific article groups.

Consequences for recycling of PVC

In recent years, growing attempts have been made to divert waste PVC from landfill and to develop systems for the collection and recycling of PVC waste. In general terms, recycling of waste PVC can be achieved either by mechanical or chemical methods. Failing that, PVC waste can in principle be used for energy recovery, but this option has negative environmental consequences due to the generation of large amounts of hazardous flue gas cleaning residues.

With regard to PVC waste types two major groups must be distinguished (Plastic Consult & COWI, 2000) as referred to in (RPA 2010):

- Pre-consumer PVC wastes are generated both in the production of final and intermediate products (production wastes) and from the handling or installation of PVC products (installation wastes). PVC pre-consumer wastes as a group are comparatively easy to recycle, since they can be collected separately in defined qualities; and

- The recycling of post-consumer wastes is generally in the form of articles (end-of life articles such as flooring, cables, pipes, windows, packaging) and hence in more or less mixed waste fractions or as a part of composite materials. Depending on the specific articles, PVC in wastes can occur as a more or less pure material fraction (in "mono fractions"), which can be extracted from the waste stream by sorting (e.g. bottles, pipes, some films, some profiles). Alternatively, PVC can form a part of composite products or materials, which must be subjected to disassembling or mechanical treatment processes in order to extract PVC (e.g. windows, car components, floorings, cables). For post-consumer wastes, the different PVC article groups determine to some extent in which specific waste stream the PVC occurs.

According to information from Recovinyl (Vinyl 2010, 2011) the registered volumes of post-consumer PVC being recycled in Europe, starting from 14,000 tonnes in 2005 to 254,814 tonnes (of which 137,679 of rigid PVC and 117,135 of flexible PVC) in 2010. The volume is more than doubled since 2007. Assuming an average content of plasticisers of 25 % the content of plasticisers in the recycle PVC is estimated to approximately 30,000 tonnes; the share of the four phthalates are unknown.

As phthalates are not used in rigid PVC a restriction on phthalates will only influence the recycling of flexible (or softened) PVC. Flexible PVC waste is recycled into powder and it is used as filler in the production of floor coverings, cables and tarpaulins. According to Recovinyl 79,310 tonnes, equivalent to 68 % of the recycled flexible PVC was used in cables.

In relation to the proposed restriction recycling of PVC waste would only be a problem if the waste contains one or more of the four phthalates either because it was produced before the restriction entered into force or because it is outside the scope of the restriction (use outdoor without contact to human skin etc). As the content of the phthalates might by about 25 % of the PVC and the concentration limit is proposed to be 0.1 %, the share of such recycled PVC in new articles might not exceed 0.4 %.

Taking the widespread use of the four phthalates and the fact that the proposed restriction will address 85 % of the use for the four phthalates (table 7) into consideration it is therefore obvious that the proposed restriction would mean that use of recycling of mixed PVC waste may be affected. At least it will not be possible to use mechanical recycling while chemical recycling might still be possible. However chemical recycling is very limited for the moment.

The price of feed materials with recycled PVC is substantially cheaper than materials with virgin PVC. RPA 2010 estimates the price to be 400 € per tonne compared to 1,200 € per tonne. However this price covers rigid as well as flexible PVC, and is reasonable to expect that the price of post-consumer flexible PVC waste is lower. With a total stop of recycled soft PVC the maximum loss of turnover would have been 46.9 million € in 2010 (€ 117,135 * 400). However, since recycled flexible PVC containing one or more of the four phthalates could still be used in cables for outdoor use (and other articles) – and since 68% of the recycled flexible PVC was used in cables according to Recovinyl - it is expected that the proposed restriction will not per se hinder recycling of flexible PVC.

It is thus important to underline that the proposed restriction will not prevent recycling of soft PVC as such.

The collecting of soft PVC waste comes from very few articles such as construction materials (flooring) and cables. Besides, not all collected soft PVC will contain one or more of the four phthalates as the alternatives (including DINP) have been used for some time. Floorings are a good example of this. In chapter B.2.2.1 is has been documented that the used amount (production in EU) of DEHP and BBP are respectively 30.000 t/y and 4.000 while the total used amount of plasticisers is 140.000-430.000 t/y (data from 2007). The import of plasticisers in floorings is around 11.000-34.000 t/y (no data on DEHP/BBP share). It is thus obvious that the share of the four phthalates is significantly smaller compared to other plasticisers.

As previous mentioned there are several uses that are not covered by the proposed restriction and the recycled PVC could be used for these purposes. One obvious example is cables for outdoor use, and already now the biggest amount of recycled PVC is used for production of cables.

According to article 33 in the REACH Regulation the supplier of an article containing one or more of the four phthalates in a concentration of more than 0.1 % shall inform the customer (upon request) if the article contains one or more of the four phthalates. This means that also the producer or importer of an article using recycled PVC have to know the content of the four phthalates in the final article. This has the consequence that the recycler will have to know whether or not one or more of the four phthalates are in the original PVC-article (unless the answer to the customer is a generic answer that the article might contains the substance in a concentration higher than 0.1 %). Knowledge on the individual articles and their (actual) content of the plasticiers is therefore something the producer/importer of articles made of recycled PVC needs to have disregarding the actual proposal.

The proposed restriction will have some consequences for how the recycled flexible PVC is used (if it contains one or more of the four phthalates). The exposure is not connected to whether or not the articles are made of new or recycled PVC, and given the consequences of exposing the population to the four phthalates as addressed in chapter B.10 - and taking the assumed limited economic consequences into consideration - it seems appropriate and in line with the principle of proportionality that the restriction also should cover recycling of PVC waste.

The question on how to address recycling of PVC is not new as there is already restrictions (with concentration limits) on substances that have been used previously in PVC. The recycling of any material is always complicated in this respect.

It is the dossier submitters view that the (3) phthalates in recycled PVC generally is covered by the authorisation process as recycled PVC is seen as a mixture (which is a part of the authorisation process according to article 56,6 of the REACH Regulation). Therefore the use also in recycling as such is seen as problematic and could only be granted authorisation if the argumentation demonstrates this need.

E.2.1.2 Assessment of practicality

The proposal for restriction concerns the placing on the market of articles containing one or more of the four phthalates. The data available seems to indicate that in those cases the four phthalates are eliminated by substitution to other plasticisers, costs of raw materials will normally be the dominant cost element to be considered, while cost elements like a change of manufacturing process and changes in production equipment seems to be of less significance for the products groups covered.

This does not necessarily mean that these cost elements are insignificant, but it indicates that costs related to change of manufacturing process and production equipment are not to considered to be an important constraint to substitution.

During consultation with industry no information has been received that substitution of the four phthalates should imply major implementation problems.

Some articles might be used outdoors as well as indoors. In order to avoid unintended indoor use of such articles they are covered by the proposal even if the actual intended use of a specific article is for outdoor use.

Therefore the proposed restriction is considered to be manageable. Three of the four phthalates are already restricted in other articles (toys and childcare articles) with the same content limit. The use of alternative plasticisers may imply some changes in processing and material composition and may require some research and development. The R&D cost of substituting e.g. DINP for DEHP is assumed to be relatively low. It is not expected that small and medium sized enterprises (SMEs) will be affected more than the general industry in the sectors in question with respect to the technical compliance. It is expected that the suppliers offering the alternatives are large companies, and they serve as general custom advisers when it comes to adjusting polymer formulations and production setup.

The placing on the market of articles containing one or more of the four phthalates will be enforced mainly by inspecting the producers, importers or retailers. The test method(s) could be the same for enforcement of the existing Annex XVII, entry 51 and 52 and toys directive. There are no harmonised or standardised methods for the measurements in the already existing legislation. This has to our knowledge however not caused any major enforcement problems, as there are test methods available, although it has to be stated that the tests themselves has some problems regarding recovery, especially if the content is very low; this could also be enlarged due to some variability in the article themselves. On the other hand the content of the four plasticisers will generally be much higher than the detection limit if they are deliberately added, as they have very little effect as plasticisers if they are only present in very low concentrations.

The analysis is carried out by means of gas chromatography with mass spectrometric detection (GC-MS), which is a well-established and common technique. There is therefore no need for development of additional test methods even though a standardisation would be preferable. There is already ongoing work in relation to developing an ISO-standard for “Determination of phthalate plasticiser in toys and children’s products”. The cost pr. measurement is app. € 190 per sample when more than 20 samples are analysed (Danish prices). The same limits used in the REACH Regulation, Annex XVII, would apply to this proposal so there are no disturbing differences between these limits as industry and enforcement people are accustomed to this limit.

The content of phthalates in articles can be measured with detection limits around 10 mg/kg or 0.001 % (m/m). The relative uncertainty of the method is generally estimated to be 10-15%.

A cheap way of getting information on the use of PVC and thereby possible use of the four phthalates is using a XRF which can detect the presence of chlorine. Of course this is in no way a method, that can confirm the use of the phthalates but it can quickly screen the articles and exclude the costly analysis of one or more of the four phthalates in a given article if it does not contain chlorine and thereby PVC.

The main extra costs are estimated to be related to control; both by the manufacturers, importers and the authorities. The presence of DEHP, BBP, DBP and DIBP cannot be determined by simple screening methods, e.g. XRF (see above for detecting PVC/chlorine); therefore sampling, extraction and laboratory analysis is required. The extra costs would therefore comprise the costs of sampling, sample preparation and analysis.

As the enforcement of the ban on the use of the three phthalates following the inclusion in Annex XIV is outside the scope of this restriction proposal only the testing activities related to imported articles is described.

For importers it might be necessary to require testing of products in order to ensure compliance of imported products of PVC. The frequency of the need to require testing depends of the market situation and the relation between the importer and the supplier. For importers with changing suppliers it will be necessary to have frequent testing. The cost pr unit will depend on the size of the batches. However in practice the importer, retailers etc. would normally rely on information from suppliers of the content of the phthalates in the articles.

It has to be ensured that all articles containing plasticised materials do not contain the four phthalates. According to ECHA 2009a, table 1-5 the tonnage of plasticised articles can be calculated to 2.5 million tonnes. The number of articles is very uncertain as the weight per item varies very much.

Furthermore the types of articles are very inhomogeneous. Some groups of articles like vinyl flooring and cables are homogeneous articles and imported in large batches, even if there might be smaller imports too. Other groups of articles are very heterogeneous and may contain many different materials, are imported in smaller quantities, and the content of the phthalates in each article can be quite low.

In any case the number of imported articles will be very large and the cost per weight unit can vary greatly between different groups of articles. It has not been possible to establish the required numbers of actual tests for imports in order to ensure compliance.

Based on the assumption that the drivers behind the administrative cost in this proposal for restrictions have similarities (rather limited substitution costs, many articles and many importers) to the drivers behind the administrative cost in the RoHS directive, it might be relevant to compare with some of the estimates in the Commission's Impact Assessment for the recast of the RoHS Directive. According to this Impact Assessment yearly administrative costs (in particular verification of compliance) make up approximately 67% of total costs, while the share of technical costs amounts to 33%. Assuming that this is also applicable for the four phthalates a rough estimate monitoring cost for imported articles would be € 6-12 million per year.

The testing cost is relevant for enforcement authorities and for importers in cases where these want to ensure that the imported articles are in compliance. Again taking the high cost of testing into consideration, the importer, retailers etc would normally rely on information from suppliers on the content.

E.2.1.3 Assessment of monitorability

As described in E.2.1.2 it is possible to measure the content of the four phthalates in articles. The outcome of the enforcement activities to that regard could be monitored on national level as well as on community level.

In addition to national reporting of enforcement success, notifications of any violation of the restrictions could be reported to the RAPEX system and could in that way be used to monitor the results of the implementation of the proposed restriction.

The costs of the monitoring in the form of compiling information from enforcement activities will be limited.

E.2.1.4 Overall assessment of the proposed restriction

The proposed restriction is considered to be proportional as an important risk can be addressed without major problems in substituting the problematic substances with less problematic alternatives in all applications identified. It also addresses recycled PVC as this to a large entend can be used for production of articles not covered by the proposal (e.g. cables for outdoor use).

It is possible to monitor and to enforce, but it has not been possible to estimate the number of tests necessary for the importers to verify that imported products comply with the restriction.

As regards toys three of the phthalates (DEHP, DBP and BBP) are already covered by restrictions in REACH, Annex XVII, entry 51, which stipulates: [DEHP, DBP and BBP] shall not be used as substances or as constituents of preparations, at concentrations higher than 0.1 % by weight of the plasticised material, in toys and childcare articles. Toys and childcare articles containing these phthalates in a concentration higher than 0.1 % by weight of the plasticised material shall not be placed on the market. Furthermore, with effect from 20 July, 2013 Directive 2009/48/EC on the Safety of Toys will ban the use of substances that are classified as carcinogenic, mutagenic or toxic for reproduction (CMR) of category 1A, 1B or 2 under Regulation (EC) No 1272/2008 in toys, in components of toys or micro-structurally distinct parts of toys unless certain specified conditions are met, and this will include all of the four phthalates.

RMO1 will therefore not touch upon the ban on articles already covered by this entry in REACH Annex XVII.

E.2.1.4.1 Justification for proposing a transitional period of 12 months

The actors need some time to adapt after a regulation has entered into force. The reasons are technical, economic, practical and regulatory.

The restriction includes a transition period enabling the market to adjust. The transition period should take depletion of stocks into account. As for the length of this transition period a balance must be struck between the need for protecting human health and the possibility for the market to adjust.

Economic aspects include considerations of the possibility for manufacturers, importers, wholesale and retail sellers not to place on the market their existing stocks of articles. On the other hand the transitional period should not be too long as this could be a pervert incitement to build up large stocks. These considerations are particularly important due to the assumption that many importers are small and medium sized companies.

Practical difficulties could be seen for importers who need to inform non-EU suppliers about the change in EU regulation.

Theoretically, the length of the transitional period could be different for different articles. However, for reasons of clarity to enforcers and to the actors who have to comply with the restrictions, there is a merit of having a common transitional period, and no valid reasons for not having only one transitional period has been detected during the making of the proposal.

When considering the length of the transitional period the health benefits should also be taken into consideration. As the articles can have a long service period it is important not to have a very long transitional period as this will prolong the exposure time for the general public because the proposal is not to ban the use but only the placing on the market of the articles.

For the above reasons a transitional period of 12 months is considered reasonable for the market operators to adapt to the requirements of the proposed restriction. A shorter period could imply implementation problems on the EU market.

E.2.1.4.2 Derogation for articles already in use

Articles containing one or more of the four phthalates already in use at the time of entry into force of the restriction need to be exempted from the ban on placing on the market. This is to ensure the sale of used (second hand) articles that were legally placed on the market prior to the proposed restriction is not criminalised and to ease the enforcement activities. As an example the restriction should not prohibit the sale of an old chair covered with imitation leather containing one of the four phthalates.

E.2.2 Restriction management option 2 (Wider scope)

In RMO2 all articles containing one or more of the four phthalates are banned. Only specifically mentioned articles, as also exempted in RMO1, e.g. on medical devices are exempted.

Table 109 contains information on which groups of articles that was not included in RMO1 but is included in RMO2.

Table 109. Extra articles covered by RMO2 compared to articles covered by RMO1

	Tonnage t/y 2007	Tonnage t/y Proportional reduction 2010	Estimated tonnage 2015
Roofing material	3,600	2,160	1,700
Wire and cables for outdoor use	12,100	7,260	5,600
Coated fabric for outdoor use	12,800	7,680	5,900
Car undercoating	4,000	2,400	1,900
Hoses and profiles for outdoor use	3,700	2,200	1,700
Lacquers and paints, adhesives and sealants	3,700	2,200	1,700
BBP articles undefined for outdoor use	600	300	200
DBP articles undefined for outdoor use	2,900	1,300	1,000
DIBP articles undefined for outdoor use	3,800	1,700	1,300
Total not in RMO1	47,200	27,200	21,000
Total in RMO1	265,100	169,800	134,400
Total in RMO2	312,500	197,200	156,500

Source: ECHA 2009c (regarding DEHP) and table 7

The effect on human health is in general expected to be same as in RMO1, although the risk via the environment is expected to be reduced to a small extend; the use of articles such as cables (to be put into the ground) is however of such a character that environmental effects seems unlikely. Furthermore it is avoided that human health are affected as a consequence of indoor use of articles that are placed on the market for outdoor use.

The risk addressed in this dossier concentrates on the human effects, and for this reason it is difficult to argue for the inclusion of articles only intended for outdoor use and without direct human skin contact disregarding the benefits in enforcement (see below).

For the environment it can be seen that the four phthalates have potential for bioaccumulation and some of them could have effects on aquatic organisms. The use as plasticiser in articles does however generally not cause environmental problems (except perhaps in very special cases). The same potential for bioaccumulation and effects can be seen for some of the alternatives, but the use does not seem generally to cause environmental problems.

According to table 109 for 2015 the total substitution requirement for RMO2 would be 156,500 tons, which is 21,900 tonnes (16 %) more than in RMO1.

The cost for substitution is estimated to € 17 – 33 million (marginal cost substitution 11-21 %), which is € 2.4 – 4.5 million more than in RMO1.

Practicality
Compared to the proposed restriction RMO 2 seems easier to manage and enforce. This is because enforcement authorities as well as the legal person placing an article on the market not will have to consider whether an article is used indoors or outdoors.

Conclusion

The costs of the RMO is regarded to be € 2.4 – 4.5 million and the scope of the restriction will be clearer as it is not needed to distinguish between articles for indoor use and for outdoor use. However no risk has been identified for outdoor use of articles (without direct long human skin contact) containing the four phthalates and therefore a ban for such articles does not seem to be justified, seen from the risk perspective. The improvement in relation to the practicability when implementing RMO2 compared to the practicability of RMO1 is not considered outweighing the costs for enforcement authorities, although the enforcement authorities as well as the legal person placing an article on the market not will have to consider whether an article is used indoors or outdoors.

E.2.3 Restriction management option 3 (specific group of articles)

This RMO limit the restrictions to specific group of articles where the content of the four phthalates have been explored (Danish EPA 2011).

- Flooring (and heavy wall covering)
- Insulation on wires and cables
- Electronic devices
- Plast coated fabric and film/sheets used for bags and brief/suitcases and similar items
- Plast coated fabrics and film/sheets used for tablecloth, curtains, shower curtains and similar items (not industrial uses)
- Carpet tiles/squares produced with (typically) PVC-foam as back cover
- Water- and air mattresses
- Plast coated wallpaper/tapestry
- Footwear
- Bathing equipment (swim-coats/wings/belts and pools - inflatable and others)
- Balls for playing and physical exercises
- Others: Sex toys, erasers

Effectiveness

Risk reduction capacity

Compared to the proposed restriction RMO1 the reduction in risk is considered to be relative smaller, as risks related to articles for use indoors or articles that could come in direct contact with skin or mucous membranes, that are not included in the list are not addressed. The reasoning behind the need for restriction is the total exposure and in general not the individual articles. It is not possible to give a precise estimate of the share of articles in RMO1 that is covered by RMO3 due to lack of information on uses not covered by the list.

Only information on the content for the two first groups is estimated. In 2007 flooring, wall covering, wires and cables contained 97,200 tons of the four phthalates. Furthermore EE devices are estimated to contain 5-20,000 tons and shoe soles are estimated to contain 19,400 tons. Hence about half of 264,000 tons has been identified. Surely the amount is higher, but probably not above 80%.

Proportionality

Economic feasibility is the same as for RMO1. However this can be said with even greater certainty because the specific articles are known. It has to be underlined that alternative plasticisers can be

used and no information has been received during elaboration of this dossier that feasible alternatives do not exist for all uses.

Cost is directly proportional with the volume of articles covered. As it is estimated to be between 50-80% the cost will be between € 8 million and € 24 million.

Practicality
Immediately RMO 3 seems easier to define as normal terminology of articles is used. However borderline cases will certainly make the manageability and especially the enforceability more difficult.

Conclusion

RMO3 will not ensure the same level of protection as RMO1, as there is no reason for exempting articles for indoor use or articles that could come in direct contact with skin or mucous membranes that are not mentioned in a specific list.

E.2.4 Restriction management option 4 (Restriction based on migration)

The risks from the four phthalates in articles are directly related to the migration of the four phthalates and not to the content per se. The migration can be to skin, mucous membranes including mouthing and to air. Therefore a RMO based on migration is considered.

The reasoning for the proposed restriction is the total exposure and in general not the individual article. The migration from the articles to air will contribute through the entire life-time of the articles, as there will be a tendency to reach equilibrium with the air (see section B.10.1.5.3 - Indoor air (gas phase and particles in air)).

In all articles where one of the four phthalates has been found and where migration test has been carried out, migration has been identified.

As the migration of phthalates in articles in general is very low[19] measured as e.g. mg/kg/hour the testing of migration to air would be quite lengthy and with relative great uncertainties in the measuring. Furthermore the migration limit should depend on the life time of the product, as there are big differences in the lifetime of products.

Tests based on migration are generally much more expensive than tests based on content. For importers and enforcement authorities this would imply a very costly procedure in order to check whether the articles comply with the restriction.

Lastly, for articles that both are used indoor and may come in contact with human skin, e.g. flooring, in principle two different tests would have to be carried out.

DEHP, DBP and BBP are also subject to restrictions in REACH Annex XVII, entry 51. According to entry 51 DEHP, DBP and BBP shall not be used as substances or in mixtures in toys and childcare articles in a concentration greater than 0.1 % by weight of the plasticised material. Furthermore toys and childcare articles containing these phthalates in a concentration greater than 0.1 % by weight of the plasticised material shall not be placed on the market.

[19] Cf. Table 3-20 in ECHA 2009C

These restrictions are also based on content and not migration (even though it is also here the migration that is the actual problem).

For these reasons a restriction based on migration does not seem feasible and appropriate.

E.3 Comparison of the risk management options

In table 110 the different elements discussed in E.2. are compared. It should be stressed that the score between different elements cannot be added. E.g. a "3" in effectiveness cannot be compared with a "3" in practicability. However the table gives an impression of the areas where the different RMOs might differ.

Table 110. Comparison of the four discussed RMOs.

Criterion	Parameter	RMO1 (proposed)	RMO2	RM03	RMO4
		Indoors use and contact exposure	All articles	Specific groups of articles	Migration based
		Score	Score	Score	Score
Effectiveness	Risk reduction capacity	3	3	2	3
	Proportionality	3	2	3	3
	Overall	3	2.5	2.5	3
Practicability	Implementability	3	3	3	1
	Enforceability	2	3	2	2
	Manageability	2	2	2	1
	Overall	2.3	2.7	2.3	1.3
Monitorability	Availability of indicators	3	3	3	1
	Ease of monitoring	1	1	1	1
	Availability of monitoring mechanisms	2	2	2	1
	Overall	2	2	2	1

Note: The score is between 1 and 3, where "3" represents the highest benefit/cost ratio.

The following conclusions are made:

- Any exposure to one or more of the four phthalates from any given article should be avoided as the existing exposure level from background exposure through indoor environment, direct contact with skin or mucous memebranes and to a lesser extend food at present constitutes an unacceptable exposure and risk.
- Furthermore no article groups have been identified where the substitution of the four phthalates is more expensive than average when the emissions of the four phthalates over the life time of the articles and the volume are taken into consideration.
- RMO2 (the wider scope) gives an overall better protection, the additional cost is limited (€ 17-35 million) but the risk for use of articles outdoors does not justify a risk (reprotoxicity) based ban.
- Limiting the restriction to only identified article groups (RMO3) seems by first glance more transparent but problems in borderline cases will without doubt arise. Furthermore there is no reason to exempt articles just because they are not mentioned in a list.
- A restriction based on migration (RMO4) is more difficult to implement – furthermore the life time of the article should then also be taken into account as well as the rise in costs for checking the articles either by importer or by enforcement authorities.

Furthermore no other community wide options are regarded to address the risk sufficiently.

Therefore only one type of restriction that will address the risks posed by the combined effect of the four phthalates, namely a total ban of articles for use indoors and for direct human contact with a concentration higher than 0.1 % by weight of the plasticised material, is considered to be relevant.

E.4 Main assumptions used and decisions made during analysis

Data for use of and alternatives to the four phthalates:
A number of assumptions have been made in the analysis, in both qualitative and quantitative terms. The present report draws significantly upon data derived from the work of consultants and their communication with industrial organisations and enterprises, where confidentiality has played a major role in getting data.

All relevant organizations were contacted, most more than once. Only a few of the contacted organizations responded with data, even though promised confidentiality. Industry organizations with a few exemptions have not been able to deliver any information at all, or if some data was delivered then the data was rather sparse (both qualitatively and quantitatively). It should however be mentioned that a few of the contacted organisations did respond with data and were very open in obtaining and providing additional information if asked.

The majority of the information from industry is thus coming from some essential players mainly in Denmark but also from a few key players on the EU market. This could make the assumptions on how the European market is somewhat biased, as the Danish Market could be a bit different from the majority of the EU Market due to the Danish companies reaction to a high concern among the Danish population as such for the use of phthalates in consumer articles.

Besides trying to obtain information from stakeholders throughout Europe, an alternative approach has been to develop the required information concerning the market and product groups based on contact to individual Danish and international companies. This approach has been adopted for the majority of the applications areas investigated. The approach, however, has some weaknesses.

The approach of developing the information required based on contact to individual Danish and international companies may to some extent suffer from difficulties in obtaining information that can be regarded as representative for European companies. As the information collected and presented especially in section B of this dossier represents either Danish companies or international companies operating in many countries, the information is not necessarily representative for all markets within EU and for all companies operating in EU.

This has some impact on, e.g. quantification of consumption of phthalates and other plasticisers within the different fields of application. While it seems possible for most product groups based on statistical data to present a reasonable estimate indicating the relevant order of magnitude for the total consumption of plasticisers within the product group considered, the data available generally does not allow for reliable estimates of the consumption of individual plasticisers. This assessment also takes into account that the statistical data at the EU level (concerning import and in particular EU manufacturing) are seldom correlated precisely with the application areas investigated.

Considering these limitations, the decision has been made not to summarize the estimates of consumption of phthalates made in the different sections and thus not to present these estimates as

very reliable. The statistics and information available is, however, presented in the relevant sections under the different applications and may be utilised with due respect.

It should also be noted that statements indicating that classified phthalates have already been more or less substituted will reflect the general trend within the EU but not necessarily be valid for all markets and companies within EU. It may well be that several smaller companies serving primarily national markets may still use classified phthalates although the general picture available is that these compounds have been substituted.

The issue of the representativeness of the data is less relevant to consider discussing availability of alternatives and trends and perspectives, as these issues do not depend on the state of art of the individual companies. However, the picture presented may be dominated by companies being relatively advanced (frontrunners).

The general way of overcoming the study weaknesses mentioned above will normally be to develop a detailed knowledge of the markets in question based on inter alia a close collaboration with the trade organisation active on the these markets. The assistance received from European trade organisations, however, has been limited and not sufficient to compensate for the study weaknesses as also stated in section B and G.

E.5 The proposed restriction and summary of the justification

Based on the justifications summarised in Section A.2 and discussed in the report the proposal is to ban the on the placing on market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing DEHP, DBP, BBP and DIBP in concentrations greater than 0.1 % by weight of any plasticised material.

In detail, the following restrictions with derogations are proposed for the placing on the market of articles intended for indoor use and articles that may come into direct contact with the skin or mucous membranes:

1) For DEHP, DBP and BBP and DIBP a ban is proposed on the placing on the market of articles intended for use indoors and articles that may come into direct contact with the skin or mucous membranes containing one or more of these phthalates in a concentration greater than 0,1 % by weight of any plasticised material.

For these substances it is furthermore proposed that:

- the restriction includes a transition period enabling the market to adjust. The transition period should take depletion of stocks into account. As for the length of this transition period a balance must be struck between the need for protecting human health and the possibility for the market to adjust (e.g. 12 months from the date of entry into force of the restriction).
- by way of derogation, the ban should not apply to the immediate packaging of medicinal products covered by Regulation (EC) No 726/2004, Directive 2001/82/EC or Directive 2001/83/EC, or to medical devices covered by Directive 90/385/EEC, Directive 93/42/EEC or Directive 98/79/EC.
- by way of derogation, the ban should not apply to toys Toys containing DEHP, DBP and BBP are covered by an existing restriction. Furthermore with effect from 20. July, 2013 Directive 2009/48/EC on the Safety of Toys will ban the use of substances that are classified as carcinogenic, mutagenic or toxic for reproduction (CMR) of category 1A, 1B or 2 under

Regulation (EC) No 1272/2008 in toys, in components of toys or micro-structurally distinct parts of toys unless certain specified conditions are met.

- By way of derogation, the ban should not apply to childcare articles in respect of DEHP, DBP and BBP. Childcare articles containing DEHP, DBP and BBP are covered by an existing restriction.
- by way of derogation, the ban should not apply to articles intended to come into contact with food covered by Regulation (EC) No 1935/2004 and specific measures under this regulation, e.g. Commission Regulation (EU) No 10/2011.
- by way of derogation, the ban should not apply to articles intended for use indoors and articles which may come into direct contact with the skin or mucous membranes which were in use in the European Union prior to the date of entry into force of the restriction.
- the restriction includes a definition of 'childcare article' corresponding to the definition in the existing restrictions in entries 51 and 52 in Annex XVII
- the term "use" is defined in the restriction as meaning any placing, keeping, storing, hanging, laying, mounting, fixing or other application indoors of articles.

Only one type of restriction will address the risks posed by the combined effect of the four phthalates, namely a total ban of articles with a concentration higher than 0.1 % by weight of the plasticised material.

The proposed restriction will result in a significantly decreased exposure of the public to the four phthalates. Still some exposure from other sources will continue, due to exempted articles emitting the four phthalates, articles on the market before the enter into force of the restriction, dust and food (as the phthalates may still be found in the environment). On the long term the exposure from most of these sources will also decline as a result of the proposed restriction as the articles

The existence of alternatives for all applications underlines the possibilities for a quick phase-out. The alternatives available may include other plasticisers as well as other types of materials. No examples have been identified of products for which alternatives are not available.

The dominant alternatives to the four phthalates reported used for the covered product groups are DINP and DIDP. For most applications, however, non-orthophthalate plasticisers such as DOTP, and other plasticiser types such as DINCH, ASE, ATBC, COMGHA and dibenzoates are actually in use, or being considered by producers as realistic alternatives to ortho-phthalates.

The most obvious alternative to the four phthalates for a large part of the articles is DINP. It has to be underlined that some experimental data indicates a potential for having the same characteristics as the four phthalates (Repr. Cat. 1B). However, it must also be underlined that the potency of DINP is much lower than for the four phthalates. No data indicates similar problems for the other main alternative DIDP.

Other Community-wide risk management options have been considered and found not efficient as it is demonstrated in section E.1.3. Other risk managements options considered is a wider scope (all articles, a narrower scope (only specific groups of articles) and a restriction based on migration instead of content.

Regarding costs, substitution of classified phthalates to DINP/DIDP seems generally to be the least expensive option, as these phthalates for most applications can substitute directly for the four phthalates with no major changes needed in the production process. The effective relative price of DINP (reflecting both the price per kg and the need for more plasticiser to obtain the same effect)

was in 2006 about 11% higher than the price of DEHP. The relative price of DIDP was about 21% higher. However, the cost of plasticisers comprises only a minor part of the total production price of the article as demonstrated in section E.2.1.2.

The proposal for restriction concerns the placing on the market of articles containing one or more of the four phthalates. In general, no problems related to the implementability have been detected as shown in section E.2.1.3. The data available seems to indicate that in those cases the four phthalates are eliminated by substitution to other plasticisers, costs of raw materials will normally be the dominant cost element to be considered, while cost elements like a change of manufacturing process and changes in production equipment seems to be of less significance for the products groups covered.

No information has been received that substitution of the four phthalates should imply major implementation problems, even though some changes in processing and material composition may require some research and development, but the R&D cost of substituting e.g. DINP for DEHP is assumed to be relatively low as shown in section E.2.1.3.

The proposed restriction is considered to be manageable. Three of the four phthalates are already restricted in other products (toys and childcare articles) with the same content limit. No major practical problems in ensuring compliance with the existing legislation have been encountered in this regard. Practicalities in defining the scope is however less in RMO2 (wider scope).

The placing on the market of articles containing one or more of the four phthalates can be enforced mainly by inspecting producers, importers or retailers.

The main extra costs are estimated to be related to control; both by the manufacturers, importers and the authorities. The presence of the four phthalates cannot be determined by simple screening methods, e.g. XRF; therefore sampling, extraction and laboratory analysis is required. The price of an analysis of DEHP, DBP and BBP in a flexible PVC is in Denmark reported to be app. € 190 per sample when more than 20 samples are analysed.

The testing cost is relevant for enforcement authorities and for importers in cases where these want to ensure that the imported articles are in compliance. However in practice the importer, retailers etc would normally rely on information from suppliers on the content.

F. Socio-economic Assessment of Proposed Restriction

F.1 Human health and environmental impacts

F.1.1 Human health impacts

The four phthalates DEHP, DBP, DIBP and BBP are all classified as toxic to reproduction in category 1B according to the CLP Regulation. These four phthalates are considered to have endocrine disrupting effects with similar mode of action.

The endocrine disrupting effects that are suspected to be relevant for humans in relation to the four phthalates are congenital malformations of the male reproductive organs, reduced semen quality, reduced male reproductive hormone levels, and changes in pubertal timing including changes in breast development. Whereas the effects on reproductive parameters may reduce the ability of

humans to reproduce themselves, unusually early puberty can have adverse effects on social behavior, psychological development, and it may shift some life-long health risks. Furthermore, based on the current knowledge of the biology of testicular cancer and breast cancer as well as of the shared risk factors of these cancers and some of the abovementioned effects it has also been speculated whether prenatal exposure to phthalates may play a role in the increasing incidence levels of these two hormone dependent cancers. In addition, a recent review of the literature on human health effects of phthalates suggests that exposure to several phthalates may affect also neurodevelopment, thyroid function and the respiratory system (Jurewicz & Hanke 2011). Effects on neurodevelopment and thyroid function may cause problems like learning disabilities, autism and attention deficit hyperactivity disorder (ADHD) in the human population. However, as a common mode of action is known for reproductive effects of phthalates, less is known about modes of action for the other effects mentioned above, and this document therefore focuses on reproductive effects. Restrictions on phthalates targeted at minimizing reproductive effects may have the added benefit of decreasing also possible effects of phthalates on e.g. cancer development, the respiratory system and development of the brain.

A minor improvement can be expected related to the working environment – The proposed restriction would mean that the production of the four substances would decrease significantly. According to ECHA 2010a the RAR discusses occupational exposure in detail, and examples of workplace air concentrations are given. The production of DEHP takes place in closed systems. However, both inhalation and dermal exposure may occur during the production of DEHP. Such exposures may occur during system leaks, drumming and filling of road and rail tankers, cleaning of tanks used for production, storage or transport, during service and maintenance, transfer, and process sampling. The main occupational exposure routes are inhalation of gaseous DEHP and liquid aerosols, and dermal uptake of liquid DEHP, vapour and aerosols.

However it is not possible to quantify the human health effect.

F.1.2 Environmental impacts

The risk addressed is in this context concentrated on the human effects. For the environment it can be seen that the four phthalates have potential for bioaccumulation and some of them could have effects on aquatic organisms. The use as plasticiser in articles does however generally not cause environmental problems (except perhaps in very special cases). The same potential for bioaccumulation and effects can be seen for some of the alternatives, but also for these substances the use does not seem generally to cause environmental problems as seen in section C.15.

F.2 Economic impacts

Substituting cost

A rough estimate of the increase in total annually raw material cost to substitute the four phathalates is based on the assumed content of the four phthalates in articles for indoor use and human skin contact in 2015 at 134,000 ton (baseline scenario) and a price increase for substitutes in the range of 11 – 21 %[20] compared to the price of DEHP. This would result in total extra cost of €15 - 30 millions per annum (DEHP price at 1 €/kg). Since the cheapest alternative plasticiser, DINP, will be a possible substitute in most applications the extra cost would be closer to 15 millions €.

[20] Table 108 in section C

The content in imported articles is estimated to 20 % implying that the extra cost in imported articles in total will be 3 – 6 mio €.

Monitoring costs
The most important administrative cost is compliance verification, which is an ongoing expense. There are few data and many uncertainties (e.g. number of articles, number of importers, batches etc.) which make it very difficult to estimate the possible administrative cost.

For articles produced within EU the cost for monitoring per article will be relatively lower than compared to the costs for monitoring of imported products. This is beacause the restriction on the three of the four phthalates due to the inclusion in Annex XIV applies on the whole supply chain, and not only on the articles.

Based on the assumption that the drivers behind the administrative cost in this proposal for restrictions have similarities (rather limited substitution costs, many articles and many importers) to the drivers behind the administrative cost in the RoHS directive, it might be relevant to compare with some of the estimates in the Commission's Impact Assessment for the recast of the RoHS Directive. According to this Impact Assessment yearly administrative costs (in particular verification of compliance) make up approximately 67% of total costs, while the share of technical costs amounts to 33%. Assuming that this is also applicable for the four phthalates a rough estimate monitoring cost for imported articles would be 6-12 million € per year[21].

For electronic devices it is estimated that the incremental cost will be less than other similar articles as electronis devices also shall conform to the ROHS regulation, especially in relation to brominated flame retardants.

SME's
As mentioned in E.2.1.2 it is not expected that small and medium sized enterprises (SMEs) will be affected more than the general industry in the sectors in question with respect to the technical compliance. It is expected that the suppliers offering the alternatives are large companies, and they serve as general custom advisers when it comes to adjusting polymer formulations and production setup.

F.3 Social impacts
None social impacts are expected as there are identified technical and economical available substitutes.

F.4 Wider economic impacts
There has not been identified any uses where substitution is not possible and due to low substitution cost all demands to which the articles that today contain the four phthalates can be met. Therefore no wider economic impacts are expected. However, according to Art. 60 (6) of REACH the consequence of a restriction as proposed is that it is not possible to grant authorisations to use the four phthalates for the production of articles covered by the restriction for the EU market.

F.5 Distributional impacts

[21] In E.2.1.2 a rough estimate for the extra cost for raw material was 22 – 44 million €/y. The market share of imported articles is estimated to about 16 % implying that the extra substation cost related to imported products will be 3.5 to 7 millio €/y. The monitoring cost would then be twice that amount.

No major distribution impacts are expected due to low substitution cost.

F.6 Main assumptions used and decisions made during analysis
See the relevant sections, especially E.4.

F.7 Uncertainties
The hazard of the four phtalates is documented as the substances are classification as reprotoxic category 1 B. It is not possible to quantify the effects specifically in relation to the phthalates.
On the cost side there are some uncertainties due to lack of data, especially on quantities. However for the extra cost in relation the price of articles for the identified groups of articles, the estimation that the substitution cost are quite low is considered to be rather certain, as the four phthalates already have been substituted and replaced by other plasticisers including other phtalates.

F.8 Summary of the socio-economic impacts

Not relevant due to the very brief analysis.

G. Stakeholder consultation

For obtaining information about the market for the different uses of the four phthalates in the different product groups contacts has been made to a number of European trade organisations including:

European Plastic Converters (EuPC)

European Council for Plasticisers and Intermediates, (ECPI) - represents the majority of European manufacturers of plasticisers, a part of The European Chemical Industry Council, (CEFIC)

European Resilient Flooring Manufacturers (ERFMI)

European Council of producers and importers of paints, printing inks and artists' colours (CEPE)

Europacable (ECBL)

European apparel and textile confederation (Euratex)

Fachverband Wasserbett, Germany

These organisations cover a broad range of the products included in this study.

The organisations were asked in to assist in providing information relevant for this study (reference is made to the study questionnaire attached as Annex 2 in Danish EPA, 2011), and some of them have kindly initiated surveys among their members. In this context inter alia a meeting with ECPI and EuPC was held on the 22 March 2010 in Brussels.

Some information has been obtained, in particular via CEPE on the issue of wallpaper, via ERFMI on the issue of vinyl flooring, via Eurocable on the issue of wires and cables and via Fachverband Wasserbett on the issue of water beds.

The consortium of DEHP manufacturers preparing data files for the registration of DEHP under the REACH regulation (DEHP REACH Consortium) has also been contacted, and they have responded as part of a joint response with EuPC and ECPI, see below.

The joint response of EuPC, ECPI and the DEHP Consortium have kindly supplied general overall trends data and a number of indicative data on specific product types. Except for the information given separately by EuPC (2010), the organisations have requested confidentiality (EuPC, ECPI and DEHP REACH consortium, 2010b), and therefore the information cannot be cited in this report.

Besides that, none of the organisations have been able to provide the detailed data types requested in the study questionnaire.

An alternative has been to develop the required information based on contact to individual Danish and international companies. This approach has been adopted for the majority of the applications areas investigated. The approach, however, have some weaknesses as discussed in section E.4.

A list of the contacted companies and organisations is listed in Annex C under the individual product groups in Danish EPA, 2011.

Besides the contacts mentioned above, the following organisations have been contacted, but have not responded.

Footwear Association of Importers and Retail Chains (FAIR)

The European Confederation of the Footwear Industry (CEC)

The only European producer of BBP, has been contacted, but has not responded.

The data presented in the dossier were obtained before the registration deadline November 30, 2010. Currently there are two producers of DBP in the EU, one planning a continued production and REACH registration and another company which will stop production by the end of 2010 and will not register its use under REACH. The first company has kindly provided information to this study.

Considering the need for confidentiality, certain specific data from individual companies have not been given with specific reference to the source. Instead, the contact to relevant companies has been documented by listing the contacted companies' names by the end of each product specific sub-section.

In addition to the listed companies, a number of companies have been contacted, but have not responded.

H. Other information

No other information has been considered necessary.

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Annex 1 – Indoor air - simulation

Simulation - data from real products

In order to get an estimate of the concentrations of the phthalates, DEHP, DIBP, DBP and BBP in indoor air, in realistic rooms furnished with real furniture/materials marketed in Denmark a simulation has been made. The simulation is based on data from tests of consumer products, performed by the Danish EPA (2001). Two types of rooms have been simulated: one children's play room and one bathroom. The data on concentration of phthalates in vinyl flooring and the wall paper is based on tests performed in 2001 (designated OLD) and 2010 (designated NEW). The test data on concentration of phthalates in the furniture/equipment placed in the rooms are based on the tests performed in 2010.

Method

The concentrations of DEHP, DIBP, DBP and BBP in the rooms, are estimated using the model described in Xu (Xu et al. 2009). Due to limitations in available data on model parameters the following simplifications are made on this model:

- only ceiling partitioning is included as a sink.
- only the material/air partition coefficient for DEHP in vinyl flooring is given (from Clausen et. al., 2007). The material/air partition coefficients of other phthalates are estimated by a modified version of the default method suggested by Weschler et. al., 2008. Weschler et al. suggest to use Raoults law to estimate air concentrations in a boundary layer above the emitting surface:

$$C_{air} = xP\frac{m}{RT} = \frac{C_{subst}}{C_{subst} + C_{rest}} P\frac{m}{RT} \approx \frac{C_{subst}}{C_{rest}} P\frac{m}{RT}$$

C_{air}	concentration of substance in a boundary layer above the emitting surface (µg/m^3)
x	mole fraction of substance in material
P	vapor pressure (mmHg)
m	molecular weight of the substance (in kg/mole)
R	R is the universal gas constant (8.314 Pa m^3 mol^{-1} K^{-1} or 0.0821 atmM^{-1} K^{-1})
T	temperature (K)
C_{subs}	concentration of the substance in the product (kg/m^3)
C_{rest}	concentration of the rest of the material in the product (kg/ m^3), all the non-phthalate molecules

From this, the material/air partition coefficient K is found as:

$$K = \frac{Pm}{C_{rest}RT}$$

As the C_{rest} is not exactly known, it is proposed to use the method as a relative method.

The material/air partition coefficient is assumed to scale as:

$$K_1 / K_2 = \frac{P_1 m_1}{P_2 m_2}$$

This equation is used to estimate the partition coefficients for DBP, BBP and DIBP between vinyl flooring and air from the partition coefficient of DEHP.

- The value of the material/air partition coefficient for vinyl flooring is used as a surrogate for wallpaper/air, artificial leather/air and plastic/air in absence of relevant values/emission measurements.

The procedure for estimating material/air partition coefficients defined above yields the following values:

Phthalate	mw (g/mol)	P (mmHg)	K
DEHP	391	1.43e-7	**2.3e11***
DIBP	278	2.7e-5	3.1e13
DBP	278	7.5e-4	8.6e15
BBP	312	5.03e-6	6.5e12

*Xu, Y., et.al. 2010

The emission in each compartment (product/material) is proportional to the difference between the concentration in air and the concentration in the material/product. Second, the air concentration is determined by the removal due to ventilation q x C.

So for example, for just floor as a source:

$$\frac{dy}{dt} = -\frac{S_{floor} \times h_m}{V_r} \times (y - \frac{C_{floor}}{K_{floor}}) - q \times y$$

Where,

y	concentration of substance in air
S_{floor}	surface area of the source (flooring)
h_m	mass transfer rate (describes diffusion over a stagnant layer of air above the surface)
Vr	volume of the room
C_{floor}	concentration of the substance in the material (flooring)
K_{floor}	partition coefficient of the substance between material and air
q	ventilation rate (air changes per hour)

For all the other sources similar terms to the one for flooring ($-\frac{S \times h_m}{V_r} \times (y - \frac{C_{floor}}{K})$)

enter the equations, so that:

$$\frac{dy}{dt} = -ddtC_{fl} - ddtC_{wp} - ddtC_s - ddtC_{bb} - ddtC_m - ddtC_w - q \times y$$

and

$$ddtC_{wp} = \frac{S_{wallpaper} \times h_m}{V_r}(y - \frac{C_{wp}}{K_{wallpaper}}) \equiv G_{wallpaper}(y - \frac{C_{wp}}{K_{wallpaper}})$$ in which

C_{wp}	concentration of substance in wallpaper
$S_{wallpaper}$	surface area if the source (wallpaper)
h_m	mass transfer rate (describes diffusion over a stagnant layer of air above the surface)
Vr	volume of the room

C_{floor} concentration of the substance in the material (flooring)
$K_{wallpaper}$ partition coefficient of the substance between wall paper material and air
q ventilation rate (airchanges per hour)
$G_{wallpaper}$ S_{floor} *x hm/ Vr*

and similar for the compartments chair covered by artificial leather (ddtCs), balance ball (ddtCbb), air mattress (ddtCm) and wall/floor/ceiling (ddtCw).

It is further assumed that all the product/air partition coefficients for the products (K_{floor}, K_{chair}, $K_{wallpaper}$, $K_{balance\ ball}$, $K_{mattress}$) are the same for each substance.

Simulation

The concentrations used in the calculations originate from material analyses performed by Danish EPA. Vinyl flooring and wall paper was analysed both in 2001 (designated OLD) and in 2010 (designated NEW). Other products like e.g. shower curtains were also analysed in 2001, but is not included in the scenarios designated OLD, as shower curtains is assumed to be changed frequently (once a year or every second year). In the old study, analyses were only performed for DEHP, DBP and BBP. Additionally, in the new studies DIBP were also included in the analysis.

Table A.1. Concentration of phthalates in consumer products used in the calculations.

Furniture or material / Phthalate	DEHP mg/kg	DBP mg/kg	BBP mg/kg	DIBP mg/kg
Old vinyl flooring	150,000	-	900	-
New vinyl flooring	325	-	113	813
Old wall paper	100,000	-	-	-
New wall paper	24	-	-	19
Air mattress	192,000	-	-	-
Chair	391,500	11	-	41
Balance ball	442,000	20.5	-	693
Shower curtain	281,500	63.3	-	91.9

The two rooms that are simulated are a children's play room and a bathroom. The children's play room is sized 4 x 2.5 x 2.75 (L x W x H) = 27.5 m3. The floor is covered with vinyl flooring and the walls are covered by wall paper, covered with a vinyl layer. It is assumed that the room is furnished with a chair, partly covered with artificial leather, a balance ball and an air mattress. The air exchange rate has been set to 0.2 times an hour (recommendation in ECHA Guidance R15, 2010).

In the other room, a bathroom, sized 2 x 2 x 2.75 (L x W x H) = 11 m3, the floor is covered by vinyl flooring and the walls with wall paper, covered with a vinyl layer. Furthermore, there is a shower curtain made of vinyl. The air exchange rate has been set to 0.5 times an hour.

The output is presented in the table below.

In the table, the "steady-state" levels in the air, of the different phthalates, related to the different indoor simulations are found.

Table A.2. "Steady-state" levels of the four phthalates.

Sources / Phthalate µg/m3	DEHP	days	DIBP	days	DBP	days	BBP	days	TOTAL
CHILDRENS PLAY ROOM Vinyl flooring, wall paper, air mattress, chair and balance ball **NEW** vinyl flooring and wall paper	0.16	150	7e-6	0	8.2e-11	1	3.2e-6	1	**0.16**
CHILDRENS PLAY ROOM Vinyl flooring, wall paper, air mattress, chair and balance ball **OLD** vinyl flooring and wall paper	0.81	150	1e-5	0	8.2e-11	1	4.4e-5	1	**0.81**
BATH ROOM Vinyl flooring, wall paper and shower curtain **NEW** vinyl flooring and wall paper	0.26	150	1e-5	0	1.5e-9	1	1.8e-6	1	**0.26**
BATH ROOM Vinyl flooring, wall paper and shower curtain **OLD** vinyl flooring and wall paper	0.8	150	6e-6	0	1.5e-9	1	2.5e-5	1	**0.8**

It should be stressed that the only difference between the OLD and the NEW scenarios is the concentration of phthalates in the vinyl flooring and in the wall paper. It may be concluded that the higher concentrations of phthalates in the air in the OLD scenarios are only caused by the the higher concentrations of phthalates in the OLD vinyl floorings and OLD wall papers. The items, with which the rooms are furnished, are the same in both the OLD and the NEW scenarios

Scenario from EU Risk Assessment Report

As a comparison to the result from the previous simulation described above, a calculation has been made by applying the method developed in the EU Risk Assessment Report on DEHP (EU RAR, 2008). In the calculation described in EU RAR the only phthalate source in the room is DEHP emitted from the vinyl flooring. Based on the results presented in table A.2 above, it is also reasonable only to take DEHP into consideration in the following calculations. Applying the EU RAR method to the data from the childrens play room, parameters like the room size, area of emitting sources, air exchange rate etc. is changed according to the following calculations:

At steady state the concentration (C) can be calculated as follows:

$$C = \frac{3{,}600 \times E \times A}{ach\ V}$$

(DEHP sources: wall paper, vinyl flooring, mattress, balance ball and chair)

E = DEHP emission rate (µg/m^2/s)	3 x 10^{-4} *
A = area of the PVC material (m^2)	48
ach = air change rate (air changes/hour)	0.2
V = volume of the room (m^3)	27.5

* (from EU RAR 2008a: Environ corporation (1988) Indoor DEHP Air Concentration Predicted after DEHP Volatilizes from Vinly Products. Prepared for Chemical Manufacturers Association). 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 x 10^{-4} and 3 x 10^{-4} ⌈g/m^2/s at 25°C was derived. The highest emission is used as a worst case in this risk assessment.

The floor is covered with vinyl flooring and the walls with wall paper containing PVC. The room is furnished with an air mattress, a balance ball and a chair partly covered with artificial leather containing PVC. The total area covered with PVC is 48 m2 and the room volume is 27.5 m3. The air exchange rate is 0.2 (recommendation in ECHAs REACH Guidance on information requirements and chemical safety assessment R.15: Consumer Exposure Estimation) DEHP concentration in air is:

$$C = \frac{3{,}600 \times 3\ 10^{-4} \times 48}{0.2 \times 27.5} = 9{,}4\ \mu g/m^3$$

This result is 10 fold higher than the concentrations found in the simulations and in the other references. In general, this may be due to the fact that the EU RAR calculation method is rough and based on very few data.

In the model used in our calculations, the emission rate (from the floor and the walls) is determined by:

hm * (Cfl/Kfloor-y1).

Note that the emission rate depends on the concentration in the room air, on time, sinks, material concentration etc., and is not a constant! This is in opposition to the assumption made in the EU RAR method. In our case the initial value of the emission is approximately 0.0025 ug/m2/s2 but it quickly drops during emission, when the room air becomes saturated. This saturation is not accounted for in the method described in the RAR, which may very well account for the fact that the calculated concentration level is 10 times higher.

In order to illustrate this, we have simulated the emission rate using our model. The emission rate is plotted on a log scale. It starts at about $10^{-3.5}$ ug/m2/s2 but drops to less than $10^{-4.5}$ug/m2/s2 after approximately 140 days, and even further after that.

Annex 2 - further information on DEHP

The following are all taken directly from EU RAR, 2008a :

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/m3) (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 F0 generation began exposure as adults (5 weeks of age) and were bred to produce the F1 generation (F1a, 1b, 1c), the F1 adults (selected from F1c weanlings) were bred to produce the F2 generation (F2a, 2b, 2c), and the F2 adults (selected from F2c weanlings) were bred to produce the F3 generation (F3a, 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 F1, F2 and F3 litters/pups until the day of necropsy (approximately 2 weeks after the last weaning). The F1, F2 and F3 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 F1c, and F2c 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 F1 generation and were terminated due to the inability to produce any F2 generation animals. A one-week cross over cohabitation was performed on the control and 10,000 ppm F1 animals (up to 17 animals/sex/group), and on the control and 7,500 ppm F2 animals (up to 17 animals/sex/group) in order to determine the affected sex. F1 and F2 animals were then paired with naive animals and received control feed during the cohabitation. Upon separation, the F1 and F2 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 F0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391 and 543 mg/kg bw/day in the F1 animals; and 0.1, 0.47, 1.4, 4.8, 14, 46 and 359 mg/kg bw/day in the F2 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)

Litter	Dose groups (ppm)							
	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 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 dorsolateral 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 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 and7,500 pm 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 at7,500 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.atro6 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 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.

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**	

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.

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 430 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 F0 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, p27kip1 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 dosedependently 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 B12

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 □-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 B12 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 325µl/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, □-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. □-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 □M, 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 doserelated. 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/m3)

(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/m3. The number of live foetuses/dam was slightly, but statistically significantly decreased in the 50 mg/m3 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 epithetlium, 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 (1 µm) 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 observed.

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 antiandrogens, 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 · 5 · 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% ± 2.1 and 4.3% ± 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 F0 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 LD50 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 LD50) 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 (14C)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 LD50) 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 14C-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/m3 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 (T4) was observed whereas the levels of serum triiodothyronine (T3) 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 T4 in saline (400 and 200 µg/kg bw/day, respectively). A vehicle group was included for each treatment group. Total concentration of T4 and T3 as well as free T3 (fT3) 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 T4, 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 T4. 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 doserelated 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 dosedependent 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 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.

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, haematoxylineosin 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 rather than causing a directc effect on the testes. Co-administration of testosterone or the vitamin B12 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/m³	no effects on male fertiliy, no testicular toxicity NOAEL 1,000 mg/m³	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 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 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 vacuolation 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/m³)	↓ number of live foetuses/dam and ↑ percentage of resorptions/dam at 50 mg/m³; the effects showed, however, no dose-response relationship NOAEL for maternal and developmental toxicity 300 mg/m³	Merkle et al. (1988)

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 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 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 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)

Annex 3 - Further information on DBP from EFSA

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food (AFC) on a request from the Commission related to

Di-Butylphthalate (DBP) for use in food contact materials

Question N° EFSA-Q-2003-192

Adopted on 23 June 2005 by written procedure

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) has been asked to re-evaluate di-butylphthalate (DBP) for use in the manufacture of food contact materials.

Previously, a temporary Tolerable Daily Intake (t-TDI) of 0.05 mg/kg bw was set by the Scientific Committee for Food (SCF), based on the endpoint of peroxisome proliferation in rodent liver. There is now a scientific consensus that liver peroxisome proliferation in rodents is not relevant for human risk assessment. The critical effects of DBP relate to reproduction. From the several studies available, the critical observations were as follows.

In a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals, the lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a Lowest Observed Adverse Effect Level (LOAEL), based on embryotoxic effects on pup weight and number of live pups per litter. These effects were seen in the absence of maternal toxicity. It should be noted that the LOAEL of 52 mg/ kg bw/day (0.1% in the diet) was derived from an extensive study utilising sensitive endpoints (such as sperm parameters, oestrous cycle characterization and detailed testicular histopathology).

In another reproduction study, a No Observed Adverse Effect Level (NOAEL) of 50 mg/kg bw/day and a LOAEL of 100 mg/kg bw/day for toxicity of DBP on male reproductive development in the F1 generation have been observed.

A recent developmental toxicity study in the rat, with dietary exposure to DBP during the period from late gestation (gestational day 15) to the end of lactation (postnatal day 21), has shown effects on the development of male and female offspring at lower doses than those found previously. Based on loss of germ cell development and mammary gland change at 20 mg/kg in the diet (the lowest tested dose), a NOAEL could not be established.
However, given the reversibility of the effects at all dose levels and especially at the lowest dose level (20 mg/kg feed, which corresponds to 1.5 to 3 mg/kg bw/day) and also given that in several reproductive toxicity studies with longer exposure periods approximately 30 -fold higher NOAELs or LOAELs have been determined, an uncertainty factor of 200, to derive a TDI for DBP based on the LOAEL of 20 mg/kg feed is considered sufficient.

According to the above statement, the Panel allocated a TDI for DBP of 0.01 mg/kg bw, based on a LOAEL of 2 mg/kg bw/day and making use of an uncertainty factor of 200.

The limited available data on DBP concentration in foods and diets in the UK and Denmark were used to provide an estimation of dietary exposure. In the UK, mean and high (97.5th percentile) intakes of DBP from dietary sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population (equivalent to 0.2 and 0.5 µg/kg bw/day) considering a 60 kg adult.
In a Danish study, DBP estimated mean exposure ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 µg/kg bw/day, considering a 70 kg adult. Based on the highest concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 µg/kg bw/day.
In a further Danish study, the main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). The total daily oral intake at the regional level (Denmark) was estimated to be 1.6 µg/kg bw/day in adults, 8 µg/kg bw/day in children aged 1 to 6 years, and 3.5 µg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae would be less than 16.4 µg/kg bw/day in infants of less than 6 months and 6.6 µg/kg bw/day in infants of more than 6 months. For infants of more than 6 months, ready-to-use baby foods were also taken into account and the exposure was therefore estimated as less than 7.9 µg/kg bw/day.

The Panel noted that exposure to DBP from food consumption is in the range of the TDI. There are, however, a number of other sources which contribute to the overall human exposure to DBP. The Panel recommends that improved estimates of exposure to DBP from all sources along with their relative importance should be provided in order to decide what proportion of the TDI can be allocated to food contact materials alone.

KEY WORDS：

Di-butylphthalate, DBP, food contact materials, CAS n° 84-74-2, FCM Ref N° 74880.

BACKGROUND

DBP may be present in food, either due to migration from food contact materials containing DBP or due to its widespread presence as an environmental contaminant which can be found in air, water, soil and food. DBP was evaluated by the Scientific Committee for Food (SCF) in 1988 when a t-TDI for use in food contact materials was established based on the then most sensitive end-point of peroxisome proliferation in rodent liver (SCF, 1995). There is a scientific consensus that liver peroxisome proliferation in rodents is not a relevant endpoint for human risk assessment (IARC, 1995). The Panel has therefore been asked to re-evaluate DBP for use in food contact materials.

TERMS OF REFERENCE

The Commission asks EFSA to re-evaluate di-butylphthalate (DBP) for use in the manufacture of food contact materials.

ASSESSMENT

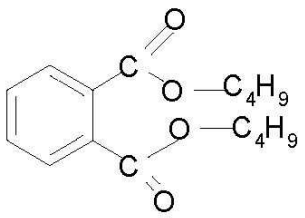
1. Chemistry

Identification of the substance

CAS-No.: 84-74-2
EINECS-No.: 201-557-4
IUPAC name: dibutylphthalate
FCM Ref N° 74880
Di-n-butylphthalate, 1,2-Benzenedicarboxylic acid, dibutyl ester (9CI), Phthalic acid, dibutyl ester (6CI, 8CI), Bis-n-butyl phthalate, Butyl phthalate, DBP, DBP (ester), Di(n-butyl) 1,2-benzenedicarboxylate, Dibutyl o-phthalate, n-Butyl phthalate, Phthalic acid di-n-butyl ester
Molecular formula $C_{16}H_{22}O_4$

Structural formula:

Molecular weight: 278.34



Purity/impurities, additives

Purity: ≥ 99% (w/w)
Impurity: ca. 0.01% (w/w) butan-1-ol
ca. 0.01% (w/w) butyl benzoate
Additives: none

Physico-chemical properties

Physical state: oily liquid
Melting point: - 69 °C
Boiling point: 340 °C at 1013 hPa
Relative density: 1.045 g/cm^3 at 20 °C
Vapour pressure: 9.7 ± 3.3 x 10^{-5} hPa at 25 °C
Water solubility: 10 mg/l at 20 °C
Partition coefficient n-octanol/water: log K_{ow} 4.572

2. Use

Based on data from industry (1995), an average of around 76% of the total DBP production is used as plasticiser in polymers, 14% in adhesives, 7% in printing inks and the remaining 3% of DBP is used in miscellaneous other applications.

3. Exposure via food

DBP is present in a large number of end products, some of which are available for consumer use. A significant use is in the food wrap or food packaging area.

No data on the levels of DBP in food in the EU attributable to migration from food contact materials have been submitted by the industry. Exposure assessment based on analytical determination of the total concentration of DBP in samples of foods or diets allow assessment of an overall dietary exposure from packaging material and other sources.

Several published data on levels of DBP in food were found in the literature. High levels (in the range of 10 to 50 mg/kg food) were reported by Castle *et al*. (1988, 1989) in confectionery and mixed dishes.

An assessment of exposure to phthalates was performed based on the analysis of stored samples from the Total Diet Study conducted in the UK in 1993. Concentration data were combined with food consumption data from the National Diet and Nutrition Study of British Adults. Since phthalates are fat soluble, only those 10 food groups which make a major contribution to dietary fat intakes were selected for analysis. Among these, carcass meat, eggs, poultry and milk were analysed for individual concentrations of phthalates since these four food groups accounted for approximately 85% of the estimated dietary intake of total phthalates. Mean and high (97.5th percentile) intake of DBP from these sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population, equivalent to respectively 0.2 and 0.5 µg/kg bw/day for a 60 kg adult (MAFF, 1996).

In a Danish study (Petersen and Breindahl, 2000), DBP was analysed in 29 different meals collected by test persons during 24 hours. Results were normalised to a daily diet of 10 MJ of energy and to a body weight of 70 kg in order to estimate total daily exposure. Among the 29 samples, 6 were above the limit of determination. The estimated mean concentration of DBP in the diet varied according to the values assigned to the 23 samples which were under the limit of determination. Estimated mean exposure therefore ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 µg/kg bw/day, considering a 70 kg adult. Based on the highest concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 µg/kg bw/day.

A further Danish assessment of DBP total dietary exposure based on estimated and measured concentrations in environmental compartments using the European Union System for the Evaluation of Substances (EUSES, a computer modeling program) was reported recently (Müller *et al*, 2003). The main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). These high contributions of vegetables to oral DBP exposure may indicate that the exposure estimates for the UK, as given above, might be underestimates of the actual exposure because the UK figures were only based on food from animal sources.
For Denmark, the total daily oral intake at the regional level was estimated to be 1.6 µg/kg bw/day in adults, 8 µg/kg bw/day in children aged 1 to 6 years, and 3.5 µg/kg bw/day in children aged 7 to

14 years. The highest local daily oral intake was estimated as 60 µg/kg bw/day in adults, 400 µg/kg bw/day in children aged 1-6 years, and 200 µg/kg bw/day in children aged 7-14 years. It must be underlined that more than 90% of these maximum exposure values derive from the highest estimated value of exposure via the local environment (printing inks) and consequently are not related to the diet itself. The contribution from dermal and inhalation exposure was negligible. Furthermore, EUSES, the computer modeling program which has been used for these intake estimates is a conservative one and the obtained values, especially for the local daily intakes, are not representative of the possible exposure via food contact materials.

In the same study (Müller *et al*, 2003), exposure from infant formulae was estimated based on two scenarios: an infant of less than 6 months weighing 5.5 kg and ingesting 900 g/day of formulae and an infant of more than 6 months weighing 8 kg and ingesting 525 g/day of formulae. DBP was measured in 11 commercial products and was below the detection limit (0.1 mg/kg of wet weight). Based on this detection limit, intake from infant formulae would be less than 16.4 µg/kg bw/day in the infant of less than 6 months and 6.6 µg/kg bw/day in the infant of more than 6 months. For infants of more than 6 months, exposure from ready-to-use baby foods was also taken into account, considering the daily consumption of one jar of 250 g containing 0.04 mg DBP/kg of wet weight (the maximum measured concentration among 11 commercial products). Total exposure from infant formulae and ready to use baby foods in infants aged more than 6 months was therefore estimated as less than 7.9 µg/kg bw/day.

4. Toxicological evaluation

Introduction

The Panel did not carry out a new extensive risk assessment but took cognisance of the previous evaluations by the SCF and in particular considered the more recent DBP European Union Risk Assessment Report (RAR), prepared for the European Union Existing Substances Regulation, 793/93, 2001 (Annex I), and the comments of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on this RAR (Annex II), in order to determine the most significant toxicological end-point for risk assessment. In addition, pivotal studies available since the RAR was published were included. Based on this information, the Panel focused on the most sensitive toxicological end-points for the evaluation of DBP, taken from the reproduction/developmental toxicity studies with this substance.

The SCF expressed its opinion on DBP in 1988 (SCF, 1995) based on the following statement in the safety data sheet:

> "Reviewing the evaluation on the phthalate esters and comparing the data on DBP with the data on di(2-ethylhexyl)phthalate, the phthalate ester studied most extensively, the Committee decided to use a safety factor of 1000 for calculating the TDI for DBP. This means that a t-TDI of 0.05 mg/kg b.w. is established".

The key toxicological aspects of DBP described in the EU RAR and the CSTEE opinion are summarized below. Further details are given in Annex 1 and 2.

> Based on the data available for DBP from a variety of genotoxicity studies and taking into consideration the non-genotoxic properties of other phthalate esters, the RAR concluded that DBP can be considered as a non-genotoxic substance. No adequate long-term toxicity and/or carcinogenicity studies on DBP are available.

The pivotal study for human risk characterisation was considered to be a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals (NTP, 1995; Wine *et al.*, 1997). The lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a LOAEL based on embryotoxic effects.

In its opinion expressed on 24-4-2001, the CSTEE agreed with the RAR conclusions outlined above and agreed that the male reproductive system is considered to be a main target of DBP toxicity. The CSTEE recommended that the more recent study performed by Mylchreest *et al*. (2000), establishing NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) values for toxicity of DBP on male reproductive development in the F1 generation as well as the earlier 2-generation rat study (NTP, 1995; Wine *et al.*, 1997) that established a LOAEL of 52 mg/kg bw/day for embryotoxicity in the F2-generation, should be used in the evaluation of the risk of reproductive toxicity.

Specific studies considered by the AFC Panel

The following studies on reproduction and developmental toxicity have been considered by the Panel for the determination of a NOAEL which could be used as a basis for a TDI calculation. Further details on these studies are given in Annex 1 (European Union Risk Assessment Report) and Annex 2 (CSTEE opinion).

In a one-generation reproduction study in mice, the NOAEL was 0.3% in the diet, equivalent to 420 mg/kg bw/day, based on effects on maternal fertility and embryotoxicity (Lamb *et al.*, 1987; Morissey *et al.*, 1989).

In one-generation reproduction studies in rats, in which females and males were exposed separately, NOAELs of 50 mg/kg bw/day in females and 500 mg/kg bw/day in males have been reported (Gray *et al.*, 1999).

In a two-generation reproduction study in rats, a LOAEL (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) based on embryotoxic effects was reported (NTP, 1995; Wine *et al.*, 1997).

In the reproduction study performed by Mylchreest *et al*. (2000), a NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) for toxicity of DBP on male reproductive development in the F1 generation have been observed.

In a recent developmental toxicity study (Lee *et al.*, 2004) with exposure during the period from late gestation (Gestational day 15) to the end of lactation on postnatal day 21 (PND 21), maternal rats were given DBP at dietary concentrations of 0, 20, 200, 2000 and 10000 mg/kg. Major results of this study are summarised below.

At PND 2, anogenital distance was significantly reduced in 10000 mg/kg male offspring. At PND 14, the incidence of retained nipples/areolae was increased in all treated male offspring compared with controls but the increase was only significant at 10000 mg/kg. At PND 21, in males, reduction of spermatocyte development as manifested by a decreased number of spermatocytes was observed from 20 mg/kg with dose-dependent increased incidence or/and severity. A significant increase in scattered foci of aggregated Leydig cells was observed at 2000 mg/kg and 10000 mg/kg. In the epididymis, significantly decreased ductular cross sections, indicating reduced coiling, were observed at 2000 and 10000 mg/kg. In the mammary glands, dilatation of alveolar buds and/or ducts was seen in male offspring from 20 mg/kg with low incidence but not achieving statistical significance in any group. In female offspring, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 20 mg/kg with a statistically significant increase at 20, 200, 2000 and 10000 mg/kg ($P<0.05$).

At postnatal week 11 (PNW 11), in males, loss of germ cell development was significant at 2000 mg/kg and above. This lesion differed markedly in severity between animals. Significant increases in vacuolar

degeneration in the mammary glands of males was present from 20 mg/kg but with similar incidence and qualitative gradation of change across the dose groups.

At PNW 20 and for the 2000 mg/kg dose, the incidence of loss of germ cell development was increased compared to controls, but without statistical significance and the change was minimal. Mammary gland lesions were observed in the untreated animals at this time point, but the incidence and/or qualitative gradation of change were increased in DBP exposed male animals with statistical significance for vacuolar degeneration at 200 mg/kg and for alveolar atrophy at 200 and 2000 mg/kg.

Thus in this study effects were noted at the lowest dose tested of 20mg/kg in the maternal diet (1.5-3.0 mg/kg bw/day). However, the loss of germ cell development was reversible at this dose, but was observed with a clear monotonic dose-dependency from 200 mg/kg (14-28 mg/kg bw/day) to 10000mg/kg (712-1372 mg/kg bw/day) at PNW 11. At PNW 20, the same effect was found but without statistical significance at the 20, 200 and 2000 mg/kg doses. Similarly, effects on the mammary gland were present in the 20 mg/kg males at PND 21 and PNW 11 but there was no significant effect at PNW 20.

CONCLUSIONS

Based on all the available toxicological evidence, the Panel concludes that effects on reproduction and development are the most sensitive end-points on which to base the risk assessment for DBP. Previous reviews have identified as pivotal several rat reproduction studies conducted in the last decade, which gave NOAELs or LOAELs in the region of 50 mg/kg bw/day, with the critical effect being on male reproductive development.

A recent developmental toxicity study in the rat (Lee *et al*., 2004), with dietary exposure to DBP during the period from late gestation (gestational day 15) to the end of lactation (postnatal day 21), has shown effects on the development of male and female offspring at lower doses than those found previously, having examined the development of reproductive tissues in considerable detail at various ages postnatally. Reduction of testicular spermatocyte development and mammary gland changes at low incidence in both sexes of offspring were seen at PND 21 at the lowest dose tested of 20 mg/kg (1.5-3.0 mg/kg bw/day) and above, with dose-dependent increased incidence or/and severity. Loss of germ cell development was no longer present at 20 mg/kg at postnatal week 11, but was still present with dose-dependency from 200 mg/kg (14-28 mg/kg bw/day) to 10000 mg/kg (712-1372 mg/kg bw/day). Non-dose-related, but statistically significant effects on the mammary gland persisted to postnatal week 11 in males at all doses, but by postnatal week 20, significant effects were only seen from 200 mg/kg and above. Based on loss of germ cell development and mammary gland changes at 20 mg/kg in the diet (the lowest tested dose), the Panel noted that a NOAEL could not be established. However, given the reversibility of the effects at all dose levels and especially at the lowest dose level (20 mg/kg feed, which corresponds to 1.5 to 3 mg/kg bw/day) and also given that in several reproductive toxicity studies with longer exposure periods only approximately 30-fold higher NOAELs or LOAELs have been determined, a safety factor of 200, to derive a TDI for DBP based on the LOAEL of 20 mg/kg feed from the Lee *et al* (2004) study is considered sufficient.

According to the above statement, the Panel allocated a TDI for DBP of 0.01 mg/kg bw, based on a LOAEL of 2 mg/kg bw/day and making use of an uncertainty factor of 200.

The limited available data on DBP concentration in foods and diets in UK (1993) and Denmark (2003) were used to provide an estimation of dietary exposure. In the UK, mean and high (97.5th percentile) intakes of DBP from dietary sources were estimated to be respectively 0.013 and 0.031 mg/person/day in the adult population (equivalent to 0.2 and 0.5 µg/kg bw/day considering a 60 kg adult).
In a Danish study (Petersen and Breindahl, 2000), the DBP estimated mean exposure ranged from 0.13 to 0.29 mg/day, i.e. 1.8 to 4.1 µg/kg bw/day, considering a 70 kg adult. Based on the highest concentration of DBP determined, exposure at high percentiles was estimated as 0.72 mg/day equivalent to 10.2 µg/kg bw/day.

In a further Danish study (Müller *et al*, 2003), the main dietary sources of exposure were estimated to be root crops (83%) and leaf crops (13%). The total daily oral intake at the regional level (Denmark) was estimated to be 1.6 µg/kg bw/day in adults, 8 µg/kg bw/day in children aged 1 to 6 years, and 3.5 µg/kg bw/day in children aged 7 to 14 years.
Based on the detection limit, intake from infant formulae would be less than 16.4 µg/kg bw/day in infants of less than 6 months and 6.6 µg/kg bw/day in infants of more than 6 months. For infants of more than 6 months, ready-to-use baby foods were also taken into account and the exposure was therefore estimated as less than 7.9 µg/kg bw/day.

The Panel noted that exposure to DBP from food consumption is in the range of the TDI. There are, however, a number of other sources which contribute to the overall human exposure to DBP. The Panel recommends that improved estimates of exposure to DBP from all sources along with their relative importance should be provided in order to decide what proportion of the TDI can be allocated to food contact materials alone.

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ANNEX 1

Extracts from the European Union Risk Assessment Report on DBP

Consolidated Final Report (dated June 2004)

Summary on mutagenicity and carcinogenicity

In assays detecting gene-mutations in bacteria, only one assay was negative in all 4 strains tested without and with metabolic activation. In two other assays, equivocal (Agarwal *et al.*, 1985) and positive (Seed, 1982).results were seen in strain TA 100 only, without metabolic activation. The positive effects were weak and seen at cytotoxic doses.
A gene-mutation test in yeast cells showed negative results (Zimmermann *et al.*, 1984).
In a mouse lymphoma assay performed only without metabolic activation, gene-mutations were induced at highly cytotoxic concentrations (NTP, 1995). An adequately performed test for gene-mutations in mouse lymphoma cells showed negative effects without metabolic activation; with metabolic activation positive effects were seen (Hazleton, 1986). In the same experiment diethylphthalate showed negative results while it is expected that, based on structure-activity relationships, mutagenic activity would increase with decreasing length of the alkyl chain. Also butylbenzyl-, di(2-ethylhexyl)-, diisononyl- and diisodecylphthalate showed negative results in the same experiment.

No chromosomal aberrations in mammalian cells were seen but the tests were performed without metabolic activation only. In one test also the induction of SCE's was studied and a slight (<2x), but statistically significant increase of SCE's was seen at all three dose-levels, but without any dose-relationship (Abe and Sasaki, 1977).

A micronucleus study performed according to current standards showed negative results (BASF, 1990). In mice exposed for 13 weeks to DBP in their diet no induction of micronuclei was observed either (NTP, 1995).
In conclusion *in vitro* studies gave an indication for a genotoxic effect in one assay, but this effect was not seen with other dialkylphthalates in the same experiment. No genotoxic effects for dibutylphthalate were observed in *in vivo* studies detecting chromosomal aberrations.

Based on the data available for DBP from a variety of genotoxicity studies as described above and taking into consideration the non-genotoxic properties of other phthalate esters, DBP can be considered as a non-genotoxic substance.

No adequate long-term toxicity and/or carcinogenicity studies in animals as well as man are available.

Summary on toxicity for reproduction and development

<u>Reproduction studies</u>

In an oral reproduction study in CD-1 mice according to a continuous breeding protocol and including the production of one generation, doses of 0.03, 0.3 and 1.0% DBP in the diet (ca. 0, 40, 420 and 1410 mg/kg

bw/day) were administered to groups of 20 m and 20 f animals for a 7d premating period, after which the animals were grouped as mating pairs and treated during a 98 day mating period. A control group of 40 m and 40 f mice received the basal diet. After the 98-days cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. At the end of the continuous breeding period a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. F_0 parents showed a significantly decreased groweighth (males only) and significantly increased liver weights (females only) at 1.0% in the diet. At 1.0% in the diet statistically significant decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive were seen. Lower dose-levels did not cause these effects. Females and not males were affected as was shown in the crossover mating trial. In this trial between control males and 1.0% females statistically significant decreases in percentage of fertile pairs, no. of live pups/litter, proportion of pups born alive and live pup weight were observed. The NOAEL for parental and embryotoxicity is 0.3% in the diet (ca. 420 mg/kg bw/day) in this study (Lamb *et al.*, 1987; Morissey *et al.*, 1989).

Gray *et al.* (1999) performed a multigeneration study in LE hooded rats. Both male and female animals (10-12 animals/sex/group) of only the P_0 generation received orally by gavage 0, 250 or 500 mg DBP/kg bw/day from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1000 mg/kg bw/day. When the P_0 animals were mated, treated animals were paired with untreated controls. F_1 animals were not treated. After puberty F_1 animals were selected (16/sex/group) for fertility assessment under continuous mating conditions over 11 breeding cycles.
In the P_0 generation delayed puberty (preputial separation) was seen in males at all dose-levels. DBP treatment did not accelerate the age at vaginal opening or induce persistent vaginal cornification, effects indicative of subchronic estrogen exposure. The P_0 generation showed reduced fertility in male and female animals at 500 and 1000 (males only) mg/kg bw/day. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated sucessfully, but many treated females (500 mg/kg bw/day) aborted their litters around midpregnancy. In the F_1 offspring which were exposed only *in utero* and lactational via dams (data only from F_1 animals from dams treated with 0, 250 and 500 mg DBP/kg bw/day), urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. In addition a few treated animals displayed anophthalmia. Furthermore F_1 males from treated mothers exhibited reduced cauda epididymal sperm numbers. The F_1 offspring showed reduced fecundity (significantly fewer F_2 pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw/day, respectively) in similarly treated pairs under continuous breeding conditions. The lowest dose-level of 250 mg/kg bw/day in this study is a LOAEL.

In an other oral reproduction study in Sprague-Dawley rats according to the continuous breeding protocol and including the production of two generations, doses of 0, 0.1, 0.5 and 1.0% in diet (0, 52, 256 and 509 mg/kg bw/day for males and 0, 80, 385 and 794 mg/kg bw/day for females) were administered to groups of 20 m and 20 f animals for a 7day premating period after which the animals were grouped as mating pairs and treated during a 112day cohabitation period. A control group of 40 m and 40 f rats received the basal diet. After the 112day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. Thereafter treatment of F1 animals was initiated at the same concentration as their parents. At the end of the continuous breeding period also a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. During the continuous breeding phase 1.0% in the diet caused a reduction in groweighth of Fo females. The total number of live pups/litter was statistically significantly decreased at all dose-levels with a dose-relationship. Live pup weights. were significantly decreased at 0.5 and 1.0% in the diet. In the crossover mating trial, designed to determine the affected sex, no effect upon mating, pregnancy or fertility indices were seen. Fo females at 1.0% showed decreased body weights. and increased rel. liver and kidney weights. Fo males at 1.0% revealed increased rel. liver-, kidney-, and right cauda epididymis weights. F1 males at 0.5% showed significantly increased kidney weights. Sperm parameters (sperm concentration and motility, % abnormal sperm or testicular

spermatid head count), estrous cyclicity, and estrous cycle were not affected. The weight of pups from treated females (1.0% in the diet) was statistically significantly decreased.

During the continuous breeding phase, after the cross-over mating trial with the F0 parents and at production of the F2 generation mating, pregnancy and fertility indices for F1 parents were statistically significantly lower at 1.0% in the diet. Live F2 pup weights. were statistically significantly lower at all dose-levels (also after adjustment for litter size). Female F1 parents at 1.0% showed statistically significantly lower body weights. and absolute organ weights (right ovary, liver, kidneys). In male F1 parents at 1.0% body weight. and rel. weights. of all reproductive organs were lower while rel. liver and kidney weights. were statistically significantly increased. Epididymal sperm count and testicular spermatid head count were statistically significantly decreased at 1.0%. Epididymides were absent or poorly developed in 12/20 F1 males at 1.0% and in 1/20 F1 males at both lower dosage levels. In 4/20 males at 1.0% and 1/20 at 0.5% in diet testicular atrophy was seen. Testes of 3/20 males at 1.0% were not descended into the scrotal sacs; 4/20 males at this dose-level had poorly developed seminal vesicles and 4/20 had an underdeveloped prepuce or penis. Histopathology showed degeneration of seminiferous tubules in 8/10 F1 males at 1.0% and in 3/10 at 0.5% DBP in the diet. 7/10 F1 males at 1.0% revealed testicular interstitial cell hyperplasia. Histopathology of seminal vesicles revealed in 1/10 F1 males at 1.0% vesiculitis with inspissated secretion. There was no indication of an effect on estrous cyclicity or duration of the estrous cycles in F1 females at all dose-levels.
In this study DBP appeared to be a reproductive toxicant in rats exposed both as adults and during development. The effects on the 2nd generation were greater than on the first generation. The lowest dose-level in this study, 0.1% in the diet (52 mg/kg bw/day for males; 80 mg/kg bw/day for females) is a LOAEL for embryotoxicity. The NOAEL for maternal toxicity is 0.5% in the diet (385 mg/kg bw/day)(NTP, 1995; Wine *et al.*, 1997).

In a fertility study female Wistar rats were exposed for 3 months before mating followed by a 7-day mating period to 0, 120 or 600 mg DBP/kg bw/day. Pregnant females were killed on day 21 of pregnancy. The study was not performed according to any guideline or under GLP conditions and the information given was limited. No maternal or embryotoxicity was seen in this study. 600 mg/kg bw/day is a NOAEL for embryotoxicity and maternal toxicity in this limited study (Nikoronow *et al.*, 1973).

In fertility studies in Charles River COBS CD rats, performed under GLP conditions, either male or female rats were exposed beginning 60 and 14 days, respectively, prior to mating, during mating, gestation and lactation. In the study in which females only were exposed, F1 weanlings were selected from all groups and were given either control diets or the same diets as their mothers for a 7 week post-weaning period (IRDC, 1984).

In the male fertility study, no effect on survival, appearance, behaviour, body weights, hematology and fertility was observed. Organ weights. of treated males showed a statistically significantly increased absolute as well as relative liver and kidney weight at 500 mg/kg bw/day. Relative kidney weights were also significantly increased in males at 50 and 5 mg/kg bw/day, but these increases were less pronounced, without a dose-relationship. Histopathology of the kidneys did not reveal abnormalities. In addition, well-performed 3 month rat studies revealed only at doses $\geq$ 350 mg/kg bw/day increased kidney weights. Therefore the increased kidney weights at 50 and 5 mg/kg bw/day seen in this male fertility study are considered as biologically insignificant. Reproductive performance, parturition, neonatal viability, growth of newborn, organ weights and histopathology in weanlings did not reveal abnormalities. The NOAEL for male fertility and embryotoxicity in this study is 500 mg/kg bw/day, the highest dose tested (IRDC, 1984).

In the female fertility study, no effect on survival, appearance, behaviour, hematology or fertility of treated females was seen. Growth of females was reduced slightly pre-mating, during the entire gestation period and during lactation period at 500 mg/kg bw/day, statistically significant at week 7, 9 and 11. At 50 mg/kg bw/day, also reductions in weight gain during the entire gestation period were seen, but less pronounced. Organ weights of treated females showed a statistically significant increase at 500 mg/kg bw/day. Histopathology did not reveal abnormalities. Reproductive performance, parturition and neonatal viability did not reveal abnormalities. Pup weight at birth and growth of pups during entire lactation period was lower at 500 mg/kg bw/day. Organ weights and histopathology of weanlings did not show abnormalities. During

the 7 week post-weaning period also reduced body weights were seen both with and without continuing treatment at all dose-levels, sometimes reaching statistical significance, but without any dose-relationship. Organ weights after 7 week post-weaning period revealed slightly decreased testicular weights in weanlings fed 500 mg/kg bw/day. After the 7 week post-weaning period, histopathology revealed testicular lesions in 6/10 weanlings (2 with mild granuloma unilateral, 1 with severe unilateral degradation, 1 with moderate bilateral degeneration, 2 with a trace of bilateral degeneration) fed 500 mg/kg bw/day. In the group derived from mothers fed 500 mg/kg bw/day and given control diet for 7 weeks post-weaning, 2/9 weanlings showed testicular lesions (1 with a trace of unilateral degeneration, 1 with severe unilateral degeneration). The NOAEL in this study is 50 mg/kg bw/day based on maternal toxicity and embryotoxicity (IRDC, 1984).

Conclusion

Male fertility of mice did not appear to be affected up to the highest dose-level of 1.0% in the diet (equivalent to 1410 mg/kg bw/day) in a one-generation study while female fertility was clearly affected at this dose-level. At 1.0% in the diet also embryotoxic effects were observed. The NOAEL in this study in mice is 0.3% in the diet equivalent to 420 mg/kg bw/day based on effects on maternal fertility and embryotoxicity.

Concerning the available reproduction studies in rats a NOAEL of 50 mg/kg bw/day can be established based on embryotoxicity in a one-generation reproduction study with exposure of females only. The same study protocol with exposure of male animals only, gave a NOAEL of 500 mg/kg bw/day.

However in a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals the lowest dose-level of 0.1 % in the diet (52 mg/kg bw/day for males and 80 mg/kg bw/day for females) appeared to be a LOAEL based on embryotoxic effects (NTP, 1995; Wine *et al*., 1997). It has to be noted (Foster, 1997) that the LOAEL of 52 mg/kg bw/day (0.1% in the diet) was derived from a more extensive study with improved sensitive endpoints (such as sperm parameters, estrous cycle characterization and detailed testicular histopathology)) compared to the study with the NOAEL of 50 mg/kg b.w. According to this author, the protocol of the continuous breeding study was supposed to identify adequately compounds with endocrine activity.

In conclusion, effects on pup weight and number of live pups per litter were seen in the absence of maternal toxicity at the lowest dose-level of 52 mg/kg bw/day in a 2-generation reproduction study in rats with a continuous breeding protocol. Other available reproduction studies in rats showed effects on fertility and embryotoxic effects at oral doses ≥250 mg/kg b.w.

Developmental studies

Developmental studies in mice and rats have been performed. None of these studies was performed according to any guideline and no data on GLP conditions were available.

ANNEX 2

Extracts from CSTEE opinions on the results of the Risk Assessment of DBP

Opinion expressed on 24-4-1998 (issued from opinion on phthalate migration from soft PVC toys and child-care articles).

Part on effects assessment

In contrast to other phthalates like DINP and DEHP, DBP is not added intentionally to soft PVC toys and child-care articles. However, DBP can be present in these toys as by-product/impurity (in trace amounts), due to the use of technical phthalate mixtures in the production process.

Similar to other phthalate esters application of DBP to laboratory animals results in peroxisome proliferation, increased liver weight, liver tumours in mice, atrophic testes, impaired fertility and embryonal development. The monoester is seen as the active toxic metabolite. There is no indication that DBP or the metabolites are genotoxic (WHO, 1997).

In the gut and liver DBP is hydrolysed to phthalic acid, n-butanol and the monobutylphthalate (White *et al*., 1983). The monoester is partially glucuronidised and excreted via the urinary tract. In rats the glucuronidation is 3-4 times lower than in hamsters, whereas glucuronidase activity in testes is higher, possibly explaining the higher sensitivity of rat testes to DBP toxicity.

Jobling *et al*. (1995) reported that DBP could reduce the binding of 17β-estradiol to the oestrogen receptor and stimulate transcriptional activity. To evaluate the relevance of these findings for the intact animal, the reproductive toxicity of DBP was studied using the NTP's Reproductive Assessment by Continuous Breeding (RACB) protocol (Wine *et al*., 1997). Levels of 0.1, 0.5 and 1.0% DBP in the diet were selected, which yielded average daily DBP doses of 52, 256 and 509 mg/kg bw/day for males and 80, 385 and 794 mg/kg bw/day for females. If the findings of Jobling *et al* (1995) were correct one would see greater reproductive effects of DBP in second generation animals, because under the RACB protocol F0 rats are exposed only as adults, whereas F1 animals are born to mothers that are treated during maturation to sexual maturity and through mating. DBP consumption by F0 rats reduced the total number of live pups per litter in all treated groups by 8-17% and live pup weights in the 0.5% and 1.0% dose groups by more than 13%. In the pups reduced number of live pups, body weights, and in the F0 animals increased kidney and liver weights have been observed at the 1% dose (509 mg/kg bw/day for males and 794 mg/kg bw/day for females). In the F1 mating trial, indices of mating, pregnancy, and fertility in the 1% dose group were decreased, concomitant with a 13% decrease in the dams body weight. Necropsy resulted in decreased epididymal sperm contents and testicular spermatid head counts. No such effects have been seen at the 0.5% dose (256 mg/kg bw/day in males, 385 mg/kg bw/day in females). The F2 pup weights were 6-8% lower in all dose groups. This study shows that the reproductive/developmental effects of DBP in the second generation were greater than on the first generation. For reproduction the NOAEL was 256 mg/kg bw/day in males and 385 mg/kg bw/day in females. In the second generation the LOAEL for reduced F2 pup weights was 52 mg/kg bw/day for males and 80 mg/kg bw/day for females.

The critical effect used for assignment of a NOAEL value for DBP is reduced F2 pup weights observed in a 2-generation reproductive study with rats (Wine *et al*, 1997). This study did not identify a NOAEL value, the LOAEL value for the critical effect was 52 mg/kg bw/day. Because the LOAEL value is used for calculating the TDI an additional uncertainty factor of 5 is used. A TDI of 100 µg/kg bw/day is assigned to DBP.

Opinion expressed on 24-4-2001

Part on effects assessment

DBP is rapidly absorbed and excreted after oral exposure. In rats dermal absorption appears to be in the range of 10% of the oral absorption. From an *in vitro* study it seems that human dermal absorption is only 2.5% of that in rat. It is the opinion of the CSTEE that this is not sufficiently discussed in the risk assessment. The CSTEE emphasises the need for more data to quantitate the probable difference in dermal absorption between humans and experimental animals. No data on absorption following inhalation exposure are available. The major part of absorbed DBP is hydrolysed to the monoester metabolite and further glucuronidated, or is subjected to oxidation leading to hydroxy and/or keto metabolites.

It is generally assumed that free MBP is the active, toxic metabolite of DBP. For the evaluation of the sensitivity of humans to DBP toxicity it is important to have information on the level of free MBP in the target tissues in humans compared to that in test animals. In the study by Blount *et al*. (2000), it was found that human urinary MBP was predominately conjugated as the glucuronide form. However, in 5% of the tested urinary samples the authors found a substantially higher concentration of unconjugated MBP.

The CSTEE agrees that the acute toxicity of DBP is low, but emphasises that the acute toxicity following inhalation exposure is difficult to assess. The CSTEE agrees that DBP is not a skin or eye irritant. However, irritation of nasal mucous membranes have been reported for mice after exposure by inhalation for 2 h to 0.25 mg/L and cats after receiving 1 mg/L for 5.5 h. Repeated exposure of rats by inhalation to concentrations = 1.18 mg/m3 induced adverse histopathological effects in the nasal cavity and larynx. These local, irritating effects in the upper respiratory tract give cause for some concern, however, due to an obvious lack of inflammation the CSTEE agrees with the conclusion of the RAR that DBP should not be classified as a respiratory irritant.

DBP has not been shown to be a skin sensitiser in well-accepted animal tests. Allergic dermatitis in humans has been reported in several studies using antiperspirants, nail polish and after contact with plastics containing DBP (watchbands, etc).
The CSTEE is aware of studies that indicate that dermal exposure to DBP may enhance the sensitisation potential of other skin sensitisers, possibly by acting as an adjuvant. DBP, in a dose-dependent fashion augmented the ability of topically applied FITC to stimulate proliferative responses in mice by draining lymph node cells, a correlate of skin sensitising potential. DBP also increased the frequency of lymph node dendritic cells bearing antigen (FITC positive DC), and increased the median amount of FITC antigen per dendritic cell. *In vitro* skin absorption studies also indicated that DBP increased the dermal absorption of FITC marginally. Exposure of mice to DBP alone did not give rise to any of the mentioned effects. The CSTEE finds that the possible adjuvant effects of DBP on other skin sensitisers should be commented upon in the RAR, and more studies on the adjuvant effects of DBP both on skin and respiratory sensitisers are warranted.

Several repeated-dose oral studies have been conducted in mice and rats. The quality of the reported studies varies, and some are not suitable for risk assessment. The key studies appear to be the NTP (1995) mice and rat studies and the rat study by Schilling *et al*. (1992). The NOAEL of 152 mg/kg/day from the Schilling study has been used in the risk assessment. In these studies changes in several haematological parameters have been used as the critical effect used to determine the NOAEL. The Schilling study is not available in the open literature (confidential study by BAYER). A more detailed description of this study in the RAR is needed in order to asses the quality of this study. It is not clear how many doses were used, from the text it could be only two doses. The NTP studies are well performed. The CSTEE recommends that the NOAEL of 177 mg/kg bw/day from the NTP rat study (based on statistical significantly decreased haemoglobin values and erythrocyte counts together with increased numbers of blood platelets) should be used as basis for the

risk assessment of systemic effects. The NTP studies, as well as several other oral studies of varying lengths, clearly show that the testis is a target tissue for DBP toxicity. In animals there are clear species differences to DBP-induced testicular toxicity. The CSTEE agrees with the RAR that the NOAEL for peroxisome proliferation is not used in the risk assessment.

An overall evaluation of bacterial mutagenicity tests shows that DBP is not a bacterial mutagen. Furthermore, no cytogenetic effects have been noted in various *in vitro* cell systems. In addition, DBP was negative in one cell transformation test. Two *in-vivo* micronucleus tests are negative. Unfortunately, most of the reported *in vitro* mutagenicity studies have not been reported in sufficient detail to allow an evaluation regarding their quality. Regarding gene mutation in mammalian cells the results are somewhat contradictory. The CSTEE has evaluated a new mouse lymphoma test (Barber *et al*., 2000) that shows a positive effect in the presence, but not in the absence of a metabolism system (S9 mix). The fact that one study showed negative effects without S9 mix (not tested with S9 mix), but two were positive in the presence of S9 mix, indicates that DBP may cause gene mutation in cells in the presence of a metabolic activation system. However, recognising the relatively high rate of false positives in the mouse lymphoma assay and the overall negative responses in all other tests, the CSTEE agrees that DBP cannot be characterised as being genotoxic.

DBP has not been tested for carcinogenicity in experimental systems, nor are there any human data available. DBP has been documented to enhance peroxisome proliferation in rats and mice. Many peroxisome proliferators have been shown to cause liver tumours when given at high doses and for long periods in mice and rats. Based on the observations that humans are non-responsive to peroxisome proliferation, the CSTEE agrees that the peroxisome proliferative effect of DBP in rats and mice is of no relevance to humans. The male reproductive system is considered to be a main target of DBP toxicity. A recent study by Mylchreest *et al*. (2000) established NOAEL (50 mg/kg bw/day) and LOAEL (100 mg/kg bw/day) values for toxicity of DBP on male reproductive development in the F1 generation. The CSTEE considers this study to be very relevant for the risk assessment of reproductive toxic effects of DBP. This study should be used together with the 2-generation rat study that established a LOAEL of 52 mg/kg bw/day for embryotoxicity in the F2-generation, in the evaluation of the risk of reproductive toxicity.

Annex 4 - Further information on DBP from EU RAR

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Reliable reproduction studies as well as developmental/teratogenicity studies in animals are summarised in **Table 4.13.**

Species	Protocol	Results
Reproduction (oral studies)		
mouse	continuous breeding protocol (one generation) 0, 0.03, 0.3 and 1.0% in diet (~0, 40, 420 and 1,410 mg/kg bw)	115 d (including 7 d premating and 98 d during cohabitation) NOAEL for embryotoxicity and parental toxicity is 0.3% in diet (~420 mg/kg bw (Lamb et al., 1987; Morissey et al., 1989)
rat	continuous breeding protocol (two generations) 0, 0.1, 0.5 and 1.0% in diet (~ 0, 52, 256 and 509 mg/kg bw for males and 0, 80, 385 and 794 mg/kg bw for females).	119 d (including 7 d premating and 112 d during cohabitation). 0.1% in diet (52 mg/kg bw for males; 80 mg/kg bw for females) is the LOAEL for embryotoxi-city. The NOAEL for maternal toxicity is 0.5% in the diet (385 mg/kg bw) (NTP, 1995; Wine et al., 1997)
rat	other; 0, 120 and 600 mg/kg bw 3 mos exposure followed by a 7d mating period	NOAEL 600 mg/kg bw for maternal toxicity and embryotoxicity (Nikoronow et al., 1973)
rat	other; 0, 5, 50 and 500 mg/kg bw via the diet to male rats only, 60 days before mating up to weaning of F1 pups	NOAEL 500 mg/kg bw with respect to fertility of male rats and embryotoxicity (IRDC, 1984)
rat	other; 0, 5, 50 and 500 mg/kg bw via the diet to female rats only, 14 days prior to mating up to weaning of F1 pups. F1 pups fed 7 weeks post-weaning	NOAEL for maternal toxicity, female fertility and embryotoxicity is 50 mg/kg bw (IRDC, 1984)
rat	other; 0, 250, 500 and 1,000 mg/kg bw exposure of P_0 generation only; two generations were produced	LOAEL 250 mg/kg bw Effects: delayed puberty in males of P_0 generation, urogenital abnormali-ties and decreased fertility of F_1 males and females (Gray et al., 1999)
Developmental toxicity (oral studies)		
mouse	other: 0, 0.005, 0.05 or 0.5% in diet (based upon food intake 0.05 and 0.5% were calculated to be 100 and 400 mg/kg bw) day 1-18 of gestation	NOAEL 0.05% in diet (100 mg/kg bw) for maternal as well as embryotoxicity and teratogenicity (Hamano et al., 1977)
mouse	other: 0, 0.05, 0.1, 0.2, 0.4, 1.0% in diet (~80, 180, 350, 660 and 2,100 mg/kg bw) day 1-18 of gestation	NOAEL for embryotoxicity is 0.2% (~350 mg/kg bw); NOAEL for maternal toxicity and teratogenicity is 0.4% (~660 mg/kg bw) (Shiota et al., 1980)
rat	other; 500, 630, 750, 1,000 mg/kg bw day 7-15 of gestation	NOAEL 500 mg/kg bw for teratogenicity. 500 mg/kg b.w is a LOAEL for maternal and embryo-toxicity (Ema et al., 1993)
rat	other; 0, 0.5, 1.0 or 2.0% in the diet (~331, 555 and 661 mg/kg bw) from day 11-21 of gestation	NOAEL 0.5% in diet (~331 mg/kg bw). Critical effect: undescended testes, decreased anogenital distance in male progeny (Ema et al., 1996)
rat	other; 0, 120 and 600 mg/kg bw day 1-21 of gestation	NOAEL 120 mg/kg bw for embryotoxicity (Nikoronow et al., 1973)
rat	other; 0, 250, 500 and 750 mg/kg bw from day 3 of gestation throughout gestation and lactation. Pups were allowed to mature.	LOAEL 250 mg/kg bw Critical effect: disturbed development of male reproductive tract (Mylchreest et al., 1998)
rat	other; 0, 500, 1,000, 1,500 and 2,000 mg/kg bw on day 14 of gestation	NOAEL 500 mg/kg bw. At doses ≥1,000 mg/kg bw higher incidences of skeletal variations. At doses ≥ 1500 mg/kg bw increased no. of resorptions and reduced fetal body wts. (Saillenfait et al., 1998)
rat	other; 0, 100, 250 and 500 mg/kg bw from day 12-21 of gestation.	LOAEL 100 mg/kg bw. Critical effect: delayed (2-days) preputial separation (one litter) (Mylchreest et al., 1999)
rat	other; 0, 250, 500 and 1,000 mg/kg bw exposure of P_0 generation only; two generations were produced	LOAEL 250 mg/kg bw for delayed puberty in males of P_0 generation, urogenital abnormali-ties and decreased fertility of F_1 males and females (Gray et al., 1999)

Reproduction studies

In an oral reproduction study in CD-1 mice according to a continuous breeding protocol and including the production of one generation, doses of 0.03, 0.3 and 1.0% DBP in the diet (ca. 0, 40, 420 and 1,410 mg/kg bw) were administered to groups of 20 m and 20 f animals for a 7-day premating period, after which the animals were grouped as mating pairs and treated during a 98-day mating period. A control group of 40 m and 40 f mice received the basal diet. After the 98-day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. At the end of the continuous breeding period a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. F0 parents showed a significantly decreased growth (males only) and significantly increased liver weights (females only) at 1.0% in the diet. At 1.0% in the diet statistically significant decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive were seen. Lower dose-levels did not cause these effects. Females and not males were affected as was shown in the crossover mating trial. In this trial between control males and 1.0% females statistically significant decreases in percentage of fertile pairs, no. of live pups/litter, proportion of pups born alive and live pup weight were observed. The NOAEL for parental and embryotoxicity is 0.3% in the diet (ca. 420 mg/kg bw) in this study (Lamb et al., 1987; Morissey et al., 1989).

Gray et al. (1999) performed a multigeneration study in LE hooded rats. Both male and female animals (10-12 animals/sex/group) of only the P0 generation received orally by gavage 0, 250 or 500 mg DBP/kg bw from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1,000 mg/kg bw. When the P0 animals were mated, treated animals were paired with untreated controls. F1 animals were not treated. After puberty F1 animals were selected (16/sex/group) for fertility assessment under continuous mating conditions over 11 breeding cycles.

In the P0 generation delayed puberty (preputial separation) was seen in males at all dose-levels. DBP treatment did not accelerate the age at vaginal opening or induce persistent vaginal cornification, effects indicative of subchronic estrogen exposure. The P0 generation showed reduced fertility in male and female animals at 500 and 1,000 (males only) mg/kg bw. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated sucessfully, but many treated females (500 mg/kg bw) aborted their litters round midpregnancy. In the F1 offspring which were exposed only *in utero* and lactational via dams (data only from F1 animals from dams treated with 0, 250 and 500 mg DBP/kg bw), urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. In addition a few treated animals displayed anophthalmia. Furthermore F1 males from treated mothers exhibited reduced cauda epididymal sperm numbers. The F1 offspring showed reduced fecundity (significantly fewer F2 pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw, respectively) in similarly treated pairs under continuous breeding conditions. The lowest dose-level of 250 mg/kg bw in this study is a LOAEL.

In an oral reproduction study in Sprague-Dawley rats according to the continuous breeding protocol and including the production of two generations, doses of 0, 0.1, 0.5 and 1.0% in diet (0, 52, 256 and 509 mg/kg bw for males and 0, 80, 385 and 794 mg/kg bw for females) were administered to groups of 20 m and 20 f animals for a 7-day premating period after which the animals were grouped as mating pairs and treated during a 112-day cohabitation period. A control group of 40 m and 40 f rats received the basal diet. After the 112-day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. Thereafter treatment of F1 animals was initiated at the same concentration as their parents. At the end of the continuous breeding period also a 7-day crossover mating trial was performed with Fo animals of control and 1% groups. During the continuous breeding phase 1.0% in the diet caused a

reduction in growth of Fo females. The total number of live pups/litter was statistically significantly decreased at all dose-levels with a doserelationship. Live pup wts were significantly decreased at 0.5 and 1.0% in the diet. In the crossover mating trial, designed to determine the affected sex, no effect upon mating, pregnancy or fertility indices were seen. Fo females at 1.0% showed decreased body wts and increased rel. liver and kidney wts. Fo males at 1.0% revealed increased rel. liver-, kidney-, and right cauda epididymis wts. F1 males at 0.5% showed significantly increased kidney weights. Sperm parameters (sperm concentration and motility, % abnormal sperm or testicular spermatid head count), estrous cyclicity, and estrous cycle were not affected. The weight of pups from treated females (1.0% in the diet) was statistically significantly decreased.

During the continuous breeding phase, after the crossover mating trial with the F0 parents and at production of the F2 generation mating, pregnancy and fertility indices for F1 parents were statistically significantly lower at 1.0% in the diet. Live F2 pup wts were statistically significantly lower at all dose-levels (also after adjustment for litter size). Female F1 parents at 1.0% showed statistically significantly lower body wts and absolute organ wts (right ovary, liver, kidneys). In male F1 parents at 1.0% body wt. and rel. wts of all reproductive organs were lower while rel. liver and kidney wts were statistically significantly increased. Epididymal sperm count and testicular spermatid head count were statistically significantly decreased at 1.0%. Epididymides were absent or poorly developed in 12/20 F1 males at 1.0% and in 1/20 F1 males at both lower dosage levels. In 4/20 males at 1.0% and 1/20 at 0.5% in diet testicular atrophy was seen. Testes of 3/20 males at 1.0% were not descended into the scrotal sacs; 4/20 males at this dose-level had poorly developed seminal vesicles and 4/20 had an underdeveloped prepuce or penis. Histopathology showed degeneration of seminiferous tubules in 8/10 F1 males at 1.0% and in 3/10 at 0.5% DBP in the diet. 7/10 F1 males at 1.0% revealed testicular interstitial cell hyperplasia. Histopathology of seminal vesicles revealed in 1/10 F1 males at 1.0% vesiculitis with inspissated secretion. There was no indication of an effect on estrous cyclicity or duration of the estrous cycles in F1 females at all dose-levels.

In this study DBP appeared to be a reproductive toxicant in rats exposed both as adults and during development. The effects on the 2nd generation were greater than on the first generation. The lowest dose-level in this study, 0.1% in the diet (52 mg/kg bw for males; 80 mg/kg bw for females) is a LOAEL for embryotoxicity. The NOAEL for maternal toxicity is 0.5% in the diet (385 mg/kg bw) (NTP, 1995; Wine et al., 1997).

In a fertility study female Wistar rats were exposed for 3 months before mating followed by a 7-day mating period to 0, 120 or 600 mg DBP/kg bw. Pregnant females were killed on day 21 of pregnancy. The study was not performed according to any guideline or under GLP conditions and the information given was limited. No maternal or embryotoxicity was seen in this study. 600 mg/kg bw is a NOAEL for embryotoxicity and maternal toxicity in this limited study (Nikoronow et al., 1973).

In fertility studies in Charles River COBS CD rats, performed under GLP conditions, either male or female rats were exposed beginning 60 and 14 days, respectively, prior to mating, during mating, gestation and lactation. In the study in which females only were exposed, F1 weanlings were selected from all groups and were given either control diets or the same diets as their mothers for a 7-week post-weaning period (IRDC, 1984).

In the male fertility study no effect on survival, appearance, behaviour, body wts, hematology and fertility was observed. Organ wts of treated males showed a statistically significantly increased absolute as well as relative liver and kidney wt. at 500 mg/kg bw. Relative kidney wts were also significantly increased in males at 50 and 5 mg/kg bw but these increases were less pronounced,

without a dose-relationship. Histopathology of the kidneys did not reveal abnormalities. In addition well-performed 3-month rat studies revealed only at doses ≥350 mg/kg bw increased kidney wts. Therefore the increased kidney wts at 50 and 5 mg/kg bw seen in this male fertility study are considered as biologically insignificant. Reproductive performance, parturition, neonatal viability, growth of newborn, organ wts. and histopathology in weanlings did not reveal abnormalities. The NOAEL for male fertility and embryotoxicity in this study is 500 mg/kg bw, the highest dose tested (IRDC, 1984).

In the female fertility study no effect on survival, appearance, behaviour, hematology or fertility of treated females was seen. Growth of females was reduced slightly pre-mating, during the entire gestation period and during lactation period at 500 mg/kg bw, statistically significant at week 7, 9 and 11. At 50 mg/kg bw also reductions in weight gain during the entire gestation period were seen, but less pronounced. Organ wts of treated females showed a statistically significantly increased relative kidney wt. at 500 mg/kg bw Histopathology did not reveal abnormalities. Reproductive performance, parturition and neonatal viability did not reveal abnormalities. Pup wt. at birth and growth of pups during entire lactation period was lower at 500 mg/kg bw. Organ wts and histopathology of weanlings did not show abnormalities. During the 7 week post-weaning period also reduced body wts were seen both with and without continuing treatment at all dose-levels, sometimes reaching statistical significance, but without any dose-relationship. Organ wts after 7-week post-weaning period revealed slightly decreased testicular weights in weanlings fed 500 mg/kg bw. After the 7-week post-weaning period histopathology revealed testicular lesions in 6/10 weanlings (2 with mild granuloma unilateral, 1 with severe unilateral degradation, 1 with moderate bilateral degeneration, 2 with a trace of bilateral degeneration) fed 500 mg/kg bw. In the group derived from mothers fed 500 mg/kg bw and given control diet for 7 weeks post-weaning, 2/9 weanlings showed testicular lesions (1 with a trace of unilateral degeneration, 1 with severe unilateral degeneration). The NOAEL in this study is 50 mg/kg bw study based on maternal toxicity and embryotoxicity (IRDC, 1984).

Conclusion on reproduction studies

Male fertility of mice did not appear to be affected up to the highest dose-level of 1.0% in the diet (equivalent to 1410 mg/kg bw) in a one-generation study while female fertility was clearly affected at this dose-level At 1.0% in the diet also embryotoxic effects were observed. The NOAEL in this study in mice is 0.3% in the diet equivalent to 420 mg/kg bw based on effects on maternal fertility and embryotoxicity.

Concerning the available reproduction studies in rats a NOAEL of 50 mg/kg bw can be established based on embryotoxicity in a one-generation reproduction study with exposure of females only. The same study protocol with exposure of male animals only, gave a NOAEL of 500 mg/kg bw.

However in a two-generation reproduction study in rats with a continuous breeding protocol and with exposure of both male and female animals the lowest dose-level of 0.1 % in the diet (52 mg/kg bw[22] for males and 80 mg/kg bw for females) appeared to be a LOAEL based on embryotoxic

[22] 52 mg/kg bw was chosen as LOAEL in order to be consistent with the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment. When asked to express its opinion on phthalate migration from soft PVC toys and child-care articles, the CSTEE identified the LOAEL of 0.1% in the diet from the 2-generation reproduction study as the most critical LOAEL for DBP and set it at 52 mg/kg bw (CSTEE, 1998). However, as the embryotoxic effects observed are more likely to occur from maternal than from paternal dosing, the LOAEL of 0.1% in the diet would in fact correspond more to 80 mg/kg bw than to 52 mg/kg bw. This can be accounted for when interpreting the MOS.

effects (NTP, 1995; Wine et al., 1997). It has to be noted that the LOAEL of 52 mg/kg bw5 (0.1% in the diet) was derived from a more extensive study with improved sensitive endpoints (such as sperm parameters, estrous cycle characterisation and detailed testicular histopathology) (Foster, 1997) compared to the study with the NOAEL of 50 mg/kg bw. According to Foster (1997), the protocol of the continuous breeding study was supposed to identify adequately compounds with endocrine activity.

In conclusion, effects on pup weight and number of live pups per litter were seen in the absence of maternal toxicity at the lowest dose-level of 52 mg/kg bw in a 2-generation reproduction study in rats with a continuous breeding protocol. Other available reproduction studies in rats showed effects on fertility and embryotoxic effects at oral doses ≥ 250 mg/kg bw. Reproduction or fertility studies with dermal exposure or exposure by inhalation to DBP are not available.

Developmental studies

Developmental studies in mice and rats have been performed. None of these studies was performed according to any guideline and no data on GLP conditions were available.

Lowest dose level administered to mice (ICR-JCL strain) was 0.005% in the diet during day 1-18 of gestation in a study of Hamano et al. (1977). Next higher dose levels were 0.05 and 0.5% in the diet (equal to 100 and 400 mg/kg bw). No. of spontaneous abortions and no. of mice with live offspring was not different from controls in any treated group. At 0.5% in the diet maternal toxicity (increased kidney wts) and embryotoxicity (lower no. of live offspring) were observed. In addition teratogenic effects were induced at 0.5% as was demonstrated by a statistically significantly higher incidence of external anomalies (non-closing eye-lid, encephalocele, cleft palate, spina bifida). Also a higher (but not statistically significantly) incidence of skeletal anomalies, especially of sternum, was seen at this dose-level. The rate of ossification was normal in all treated groups. The NOAEL for maternal, teratogenic and embryotoxic effects in this study is 0.05% in the diet equal to 100 mg/kg bw (Only summary available).

In a study of Shiota et al. (1980) mice (ICL-ICR strain) received 0.05, 0.1, 0.2, 0.4 or 1.0% DBP in their diet (ca. 80, 180, 350, 660 and 2,100 mg/kg bw) during day 1-18 of pregnancy. Maternal growth was statistically significantly reduced at 1.0%. Fetal mortality and no. of resorptions were increased at dose-levels from 0.1% onwards, but statistically significant at 1.0% only and without any dose-relationship. No. of corpora lutea and implantations were normal. Fetal wts were decreased in all treated groups, but statistically significant at 1.0 and 0.4% only. In al treated groups the incidence of skeletal variations was higher (lumbar ribs) and ossification was statistically significantly retarded as shown by the lower number of ossified coccygia. The effect on the fetal weights at the lower three dose-levels and the effect on the incidences of skeletal variations at all dose-levels can be attributed to the relatively low litter size in the control group. Limited evidence for teratogenicity was seen in this study at 1.0%. At this dose-level only 2 male and 1 female fetus survived and 2 out of these 3 survivors showed exencephaly. The dose-level of 0.2% in the diet (ca. 350 mg/kg bw) is a NOAEL with respect to embryotoxicity. For maternal toxicity and teratogenicity 0.4% in the diet (ca. 660 mg/kg bw) is a NOAEL. It has to be noted that a low number of litters was evaluated in this study and that the documentation of this study was limited.

In a developmental study in Wistar rats, 500, 630, 750 or 1,000 mg DBP/kg bw was given by gavage during day 7-15 of pregnancy. A dose relatedly increased incidence of animals with reddish-

brown staining of facial fur and piloerection was seen. Maternal death (2/11) occurred at 1,000 mg/kg bw. Maternal body weight gain was decreased at all dose-levels with a doserelationship, statistically significant at doses of 630 mg/kg bw and higher. Food consumption showed a statistically significant decrease during gestation at 750 and 1,000 mg/kg bw. No. of implantations/litter was normal. Complete resorption of implanted embryos was seen in all animals at 1,000 mg/kg bw and in 10/12 at 750 mg/kg bw. At 630 and 500 mg/kg bw 2/12 and 2/11 litters, respectively, were completely resorbed. In control group none of the litters was resorbed. Statistically significantly higher numbers of resorptions and dead fetuses/litter, higher incidences of postimplantation loss/litter and statistically significantly lower numbers of live fetuses/litter were noted at doses of 630 mg/kg bw and above. At 500 mg/kg bw, no. of resorptions and dead fetuses/litter and postimplantation loss were still increased but not statistically significant. Also the number of live fetuses/litter was still lower at 500 mg/kg bw but not statistically significant. Statistically significantly lower fetal wts were seen at 750 and 630 mg/kg bw and also at 500 mg/kg bw the fetal wt. was lower but not statistically significant. The incidences of fetuses with external malformations were higher at 630 and 750 mg/kg bw, statistically significant at 750 mg/kg bw. Cleft palate was predominantly observed. The number of fetuses with skeletal malformations was higher at 630 mg/kg bw, but not statistically significant (predominantly fused sternebrae and cervical vertebral arches). At 750 mg/kg bw too few fetuses were available for skeletal examination. 500 mg/kg bw is a LOAEL in this study for maternal toxicity and embryotoxicity. For teratogenic effects 500 mg/kg bw is a NOAEL (Ema et al., 1993).

In a follow-up study by Ema et al. (1994) pregnant Wistar rats received oral doses (by gavage) of 750, 1,000 or 1,500 mg DBP/kg bw during day 7-9, 10-12 or 13-15 of gestation. Dams were killed on day 20 of pregnancy. Postimpantation loss was 100% at 1,500 mg/kg bw at each dosing period. At 750 and 1,000 mg/kg bw post implantation loss was significantly increased regardless of the dosing period. No teratogenicity was seen at treatment during day 10-12. Treatment on day 7-9 with 750 and 1,000 mg/kg bw caused a significant increase in number of skeletal malformations (deformity of vertebral column in cervical and thoracic regions and of ribs), but neither external nor internal malformations. Treatment on day 13-15 with 750 or 1,000 mg/kg bw caused a significant increase in the incidence of fetuses with external and skeletal malformations such as cleft palate and fusion of the sternebrae. The frequency of malformations showed a doserelationship.

Ema et al. (1997b) reported complete resorption of 9/10 litters at dosing via food at a level of 2% in the diet (~895 mg DBP/kg bw) to pregnant Wistar rats on day 0-11 of gestation. Furthermore post-implantation loss/female was 98.7%. The dams showed significantly decreased weight gains and food intake.

Ema et al. (1997a) also carried out a serial study to identify the critical periods for skeletal and external malformations in rats. The animals received a single oral dose of 1,500 mg DBP/kg bw in olive oil on one of gestational days 6-16. The dams were killed on day 20 of gestation. No maternal death was seen. Maternal body weight gains in the 2-days immediately after dosing were significantly decreased. Maternal body weight gain over 0-20 days of gestation given DBP on day 6 or on one of days 6-13 was significantly decreased. The net weight gain of the dams (body weight minus gravid uterine weight) and the food intake were sigificantly decreased when DBP was given on day 16 of pregnancy. Post-implantation losses were significantly increased at dosing of dams on one of gestational days 6-16, except for days 7 and 11. Decreased fetal weights were observed at dosing on gestational days 6, 7, 8, 9, 10 (only females), 11 or 15 but not at dosing of dams on days 12, 13, 14 or 16. Significant increases in the incidences of fetuses with skeletal malformations, of fetuses with skeletal and internal malformations and of fetuses with external and skeletal malformations were observed after dosing on day 8, on day 9 and day 15, respectively. Deformity of cervical vertebrae was seen frequently after dosing on day 8. Deformity of cervical and thoracic

vertebrae and ribs and dilatation of the renal pelvis were seen predominantly after dosing on day 9. Cleft palate and fusion of the sternebrae were exclusively seen after dosing on day 15.

In a study of Sallenfait et al. (1998) pregnant Sprague-Dawley rats received a single oral dose of 0, 500, 1,000, 1,500 or 2,000 mg DBP/kg bw on day 14 of gestation. The dams were killed on day 21 of gestation. Maternal body weight gain and gravid uterine were decreased, statistically significant at 1,500 and 2,000 mg/kg bw. Increased incidences of resorptions and reduced fetal fetal body weights were observed at 1,500 and 2,000 mg/kg bw. A decreased number of live fetuses per litter was observed at 2,000 mg/kg bw. At doses ≥1,000 mg/kg bw higher incidences of skeletal variations were found. No post-implantation losses were seen in this study. The lowest dose level of 500 mg/kg bw was a NOAEL in this study.

In a study by Nikoronow et al. (1973) groups of 10 pregnant Wistar rats received for the first 21 days of pregnancy 0, 120 or 600 mg DBP/kg bw in olive oil by gavage. At 600 mg/kg bw number of resorptions was statistically significantly increased and number of fetuses and fetal wt showed statistically significant decreases. Number of dead fetuses and incidences of skeletal malformations were not affected. Placental wt was statistically significantly decreased at both 120 and 600 mg/kg bw. 120 mg/kg bw is a NOAEL for embryotoxicity in this limited study.

In a recent developmental study in rats the effects of DBP on prenatal and early neonatal development of the reproductive tract were examined. Groups of 10 pregnant CD rats (Sprague-Dawley) received by gavage 0, 250, 500 or 750 mg DBP (purity 99.8%)/kg bw in corn oil from gestation day 3 throughout pregnancy and lactation until the offspring were at postnatal day 20 with a 2-day interruption at parturition and on the following day (postnatal day 1-2). Dams were killed at weaning (postnatal day 21). Pups were killed at sexual maturity (postnatal day 100-105).

Observations

Dams were examined daily for clinical signs. Body weights were recorded daily and food consumption weekly. Dams that died or were euthanized intercurrently, were submitted to gross pathological examination, including the uterine contents (gross external examination and number of live and dead fetuses, resorptions, implantation sites). Dams which were killed on postnatal day 21, were weighed. Organ weights (ovary, uterus, liver, kidneys) of the dams were determined and post implantation sites were counted.

On postnatal day 1 live pups were counted and examined for clinical signs of toxicity, and mortality was recorded. Anogenital distance of the pups was measured. Pups were grouped by sex according to anogenital distance, and weighed. During lactation period, pups were weighed weekly in groups (by sex and litter) and examined for external abnormalities. At weaning pups were housed in groups of 3-5 animals according to treatment and sex. Individual pup weights were recorded weekly. Vaginal opening was monitored daily from postnatal day 29 until each animal acquired the developmental landmark or postnatal day 48, whichever came first.

Beginning at the onset of vaginal opening, daily vaginal lavage was conducted for 2 weeks. Males were examined for preputial separation from postnatal day 38 until acquisition. During this period, animals were also inspected for scrotal testes and hypospadias. After killing at sexual maturity (age 100-105 days) post mortem examination was conducted on all male and 3 female offspring/litter. Body and organ weights (liver, kidneys, adrenals, testes, seminal vesicles, epididymides, prostate, uterus, ovaries), position of testes, and gross morphology of internal and external genitalia were noted. Histopathology of the testes was conducted on all rats with gross lesions of the reproductive

organs and on up to 2 gross morphologically normal animals per litter per dose group. Sperm motility was determined in the right cauda epididymis (if missing, sperm analysis was not conducted).

Results

Maternal body weight and food consumption were not affected. 3 Females at 750 mg/kg bw and one at 500 mg/kg bw were not pregnant and had no implantation sites. Since pregnancy is typically achieved in approximately 85-90% of mated females, this apparent decrease may be due to the random assignment of successfully mated females among treatment groups but could also be due to preimplantation loss since dosing began on day 3 of gestation, before implantation (on day 5-6 of gestation). Uterine weight was decreased at 500 and 750 mg/kg bw, but without any dose-relationship (significant at 500 mg/kg bw only). At 750 mg/kg bw the number of live pups per litter at birth was decreased significantly. During the second half of the pregnancy body weight gain of the dams at this dose-level was slightly lower which is consistent with the smaller litters. No reduction in implantation sites on postnatal day 21 was observed at this dose-level. No effects on the proportion of pups born alive, their weights, and sex ratio were observed. Pup weight during lactation and pup weight at weaning and beyond were also not affected. Pup survival to weaning was decreased significantly at 750 mg/kg bw but survival from weaning to killing on postnatal day 100-105 was not affected.

In male offspring at birth anogenital distance was decreased at 500 and 750 mg/kg bw and at sexual maturity a dose-dependent increase in the incidence of malformations of internal and external genitalia was observed at all dose-levels. Hypospadias were observed in 3, 21 and 43% of males at 250, 500 and 750 mg/kg bw, respectively. Underdeveloped or absent epididymis, frequently bilaterally, was observed in 9, 50 and 70% of the males at 250, 500 and 750 mg/kg bw, respectively, and was associated with atrophy of seminiferous tubules (50-100% of tubules affected in all treated groups) and abnormal or reduced spermatogenesis. At 500 and 750 mg/kg bw seminal vesicles were not developed or their weight was decreased by 16 and 32%, respectively. Mean weight of the prostate gland was decreased by 27% at 750 mg/kg bw. One animal from each of 500 and 750 mg/kg group had no prostate at postmortem examination. An increased incidence of dilated Renal pelvis was observed in male offspring at all dose-levels. Mean kidney weight was significantly decreased at 750 mg/kg bw.

In female offspring DBP treatment had little effect on development of the reproductive system. At 500 mg/kg bw 1/30 rats (1/8 litters) and at 750 mg/kg bw 2/9 rats (1/4 litters) had no vaginal opening. Besides these animals, no significant changes in the age at vaginal opening and first estrus, the length of the estrous cycle, and the frequency of cornified smears in the treated groups were observed. At necropsy the rat without a vaginal opening at 500 mg/kg bw, had no patent vagina, no uterus and no left kidney. In another rat at 500 mg/kg bw right uterine horn was half of the size of the left. In one female at 750 mg/kg bw the length of the left horn was normal, but only the distal segment of the right horn near the ovary was present. A NOAEL cannot be established in this study. The results of this study suggested that DBP does not possess estrogenic activity but rather shows antiandrogenic activity at these dose-levels (Mylchreest et al., 1998).

In a follow-up study of Mylchreest et al. (1999) DBP was shown to disrupt the androgenregulated male sexual differentiation during prenatal exposure, without interacting directly with the androgen receptor, as does flutamide, a known antiandrogen. At the highest dose-level of 500 mg/kg bw (in corn oil), given orally by gavage to pregnant rats during day 12-21 of gestation, one dam showed weight loss after day 18 of pregnancy and delivered dead and moribund fetuses. At all dose levels (100, 250 and 500 mg/kg bw) delayed preputial separation in F1 males (killed at sexual maturity at

the age of 100-105 days) was seen. At the lowest dose level of 100 mg DBP/kg bw this delay (of 2 days) was attributable at least in part, to one markedly affected litter. Furthermore malformations of the (F1) male reproductive tract were observed at 250 and 500 mg/kg bw, i.e. retained thoracic nipples and decreased anogenital distance. In addition, at 500 mg/kg bw hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of seminiferous epithelium and interstitial cell hyperplasia (5 animals from 2 litters) of the testis were seen. Interstitial cell adenoma occurred at 500 mg/kg bw in 2 males (in one litter). In F1 females no abnormal uterine or vaginal development or kidney agenesis were seen. In contrast to flutamide, DBP caused a low incidence of prostate agenesis and hypospadias with no vaginal pouch.

Gray et al. (1999) reported that DBP administered orally (500 mg/kg bw) to LE hooded pregnant rats during day 16-19 of gestation reduced anogenital distance in male progeny (killed at the age of 9 months), induced retained nipples and permanently reduced androgen-dependent tissue weights. When 500 mg DBP/kg bw was given orally on gestation day 14 to lactational day 3 to SD pregnant rats again altered sexual differentiation was seen in male progeny (killed at the age of 6 months) and the effects were more pronounced than in LE hooded rats exposed for 4 days (day 16-19 of gestation).

Gray et al. (1999) also performed a multigeneration study in LE hooded rats. Both male and female animals (10-12 animals/sex/group) of only the P0 generation received orally by gavage 0, 250 or 500 mg DBP/kg bw from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1,000 mg/kg bw. When the P0 animals were mated, treated animals were paired with untreated controls. F1 animals were not treated. After puberty F1 animals were selected (16/sex/group) for fertility assessment under continuous mating conditions over 11 breeding cycles.

The P0 generation showed reduced fertility in male and female animals at 500 and 1,000 (males only) mg/kg bw. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated successfully, but many treated females (500 mg/kg bw) aborted their litters around midpregnancy. In the F1 offspring (data only from F1 animals from dams treated with 0, 250 and 500 mg DBP/kg bw) urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. In addition a few treated animals displayed anophthalmia. Furthermore F1 males exhibited reduced cauda epididymal sperm numbers. The F1 offspring showed reduced fecundity (significantly fewer F2 pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw, respectively) in similarly treated pairs under continuous breeding conditions. The lowest dose-level of 250 mg/kg bw in this study is a LOAEL.

In a study by Ema et al. (1998) pregnant Wistar rats received a diet with 0, 0.5, 1.0 or 2.0% DBP (~0, 331, 555 or 661 mg/kg bw, respectively) during day 11- 21 of gestation. The dams were killed on day 21 of pregnancy. Body weight gain and food consumption of dams during treatment period was decreased significantly at 1.0 and 2.0% DBP in the diet with a doserelationship. No post implantation loss, no changes in number of live fetuses, number of resorptions or number of dead fetuses were seen. At 2.0% weights of male and female fetuses were significantly decreased. An increased incidence of fetuses with cleft palate and fusion of the sternebrae were seen at 2.0% in the diet. At 1.0 and 2.0% in the diet the number of male fetuses with undescended testes (internal malformation) and decreased anogenital distance was increased. Anogenital distance of female fetuses in the treated groups was comparable to control values. The NOAEL in this study is 0.5% DBP in the diet (~331 mg/kg bw).

Conclusion on developmental studies

Developmental studies in rats and mice have been performed. For several studies it is unclear whether they were performed according to a guideline or under GLP conditions. Embryotoxic as well as teratogenic effects were observed. In a study in mice the dose-level of 0.05% in the diet, equivalent to 100 mg/kg bw, was a NOAEL for maternal toxicity, embryotoxicity and teratogenicity. In a second study in mice 0.2% in the diet (ca. 350 mg/kg bw) was a NOAEL for embryotoxicity; in this last study the NOAEL for maternal toxicity and teratogenicity is 0.4% in the diet (ca. 660 mg/kg bw). In this study there is a limited evidence for teratogenicity at 1.0% in the diet (ca. 2100 mg/kg bw) in the presence of maternal toxicity. However this second study showed limitations regarding the number of animals and reporting.

In several recent developmental studies in rats delayed preputial separation and a markedly disturbed development of the male reproductive tract (internal and external) of rat offspring exposed via their mothers during gestation or during gestation and lactation, was observed at oral doses ≥ 250 mg/kg bw. Maternal toxicity was seen at oral doses ≥500 mg/kg bw. In female offspring sporadic cases of reproductive tract malformations were observed at doses ≥ 250 mg/kg bw. Age at vaginal opening and estrus cyclicity were not affected. At the lowest oral dose level of 100 mg DBP/kg bw, studied in developmental studies in rats, still delayed preputial separation in male progeny was seen. The results of these studies indicate that DBP does not possess estrogenic activity but rather shows antiandrogenic activity. A NOAEL could not be derived from the available developmental studies in rats.

Developmental studies with dermal exposure or exposure by inhalation to DBP are not available.

Estrogenic activity

Recently (i.e. the last few years) concern has been raised about the possible estrogenic activity of environmental contaminants among which the phthalate esters. During the last two years several studies on this subject have been published in which many environmental contaminants have been examined for a possible estrogenic activity in a number of *in vitro* assays. The relevance of positive effects detected in these assays to human health has not yet been established.

Dibutyl phthalate was tested for possible estrogen activity *in vitro* in two human breast cancer cell lines, i.e. ZR-75 and MCF-7, by Jobling et al. (1995). DBP showed mitogenic effects on cell growth of ZR-75 cells. The growth response was less than the responses shown by β-estradiol and octylphenol. DBP also stimulated transcriptional activity of the estrogen receptor as seen in an assay with transiently transfected MC-7 cells. In addition DBP increased the transcriptional activity of the receptor in the presence of 10-11M 17β-estradiol.

Harris et al. (1997) found estrogenic activity for DBP in an *in vitro* recombinant yeast screen. The potency of DBP was estimated to be 10-7 the potency of 17β-estradiol. In addition Harris et al. (1997) found DBP to be also mitogenic in human breast cancer cells (ZR-75 and MCF-7).

Zacharewski et al. (1998) also investigated *in vitro* the estrogenic activity of DBP 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. DBP was able to compete with 17β-estradiol for binding to the rat uterine estrogen receptor *in vitro*. However the affinity for the estrogen receptor was weak. In MCF-7 cells DBP revealed weak induction of estrogen receptor-mediated gene expression while in HeLa cells no estrogen receptor-mediated activity was exhibited. In the recombinant Saccharomyces cerevisiae yeast strain PL3 DBP showed weak estrogenic activity.

The *in vivo* studies did not show reproductible, dose-dependent increases of uterine weight or cornification of vaginal epithelial cells by DBP. Gray et al. (1999) also did not find an estrogenic effect of DBP *in vivo* in a 3-day uterotropic and sex behaviour (lordosis) assay in adult ovariectomized rats with subcutaneous doses of 200 or 400 mg DBP/kg bw/day or oral gavage doses of 1,000 mg/kg bw/day administered for 2 days and followed on the third day by subcutaneous administration of 0.5 mg progesterone.

Conclusion on estrogenic activity

In some special *in vitro* assays DBP showed weak estrogenic activity. The estrogenic effects were not confirmed in *in vivo* studies. Therefore the relevance of the effects observed *in vitro* for the *in vivo* estrogenic activity of DBP is questionable.

4.1.2.9.2 Studies in humans

In a cross-sectional investigation 189 women working in processes involving DBP exposure, were examined gynaecologically. DBP concentrations exceeded 0.5 mg/m3 but quantitative data were not given and also exposure to a variety of other unspecified compounds took place. Data on a control group were not specified. An indication was found for induction of hormonal changes reflected in reduced fertility and changes in the vaginal cycle (only summary available) (Aldyreva et al., 1975).

Conclusion on studies in humans

The epidemiological study on possibly reproductive effects in occupationally exposed women showed several limitations including lack of an appropriate control group, small size of the exposed population, lack of adequate documentation of protocol and results and mixed exposure to other compounds than DBP. Therefore this study is inadequate for assessment of reproductive effects caused by DBP in humans in the working environment.

4.1.2.9.3 Conclusion on toxicity for reproduction

Concerning reproduction, fertility as well as developmental studies a NOAEL of 50 mg/kg bw can be established based on embryotoxicity in a one-generation reproduction study in rats with exposure of females only. However, a LOAEL of 52 mg/kg bw can be established based on embryotoxic effects in rats in the absence of maternal toxicity in a two-generation reproduction study with a continuous breeding protocol including improved sensitive endpoints (such as sperm parameters, estrous cycle characterisation and detailed testicular histopathology) and with exposure of both male and female animals. The protocol of this study was supposed to adequately identify compounds with endocrine activity. Therefore the LOAEL of 52 mg/kg bw will be used for risk assessment.

Based on the available developmental studies in mice an oral NOAEL of 100 mg/kg bw, can be derived for teratogenicity, embryotoxicity and maternal toxicity. At the next higher dose-level of 400 mg/kg bw embryotoxic and teratogenic effects were seen in the presence of maternal toxicity.

In rats developmental studies with exposure during gestation or during gestation and lactation, revealed delayed preputial separation and reproductive tract malformations in male offspring at oral doses ≥ 250 mg/kg bw. Maternal toxicity was seen at doses ≥ 500 mg/kg bw. At the lowest oral dose-level of 100 mg DBP/kg bw, studied in developmental studies in rats, still delayed preputial separation in male progeny was seen. A NOAEL could not be derived from the developmental studies in rats.

No reproduction, fertility or developmental studies with dermal exposure or exposure by inhalation to DBP are available.

In some special *in vitro* assays DBP showed weak estrogenic activity. However, the estrogenic effects were not confirmed in *in vivo* studies. Therefore the relevance of the estrogenic effects observed *in vitro* for the *in vivo* estrogenic toxicity of DBP is questionable. Moreover results of developmental studies described above were indicative of an antiandrogenic effect of DBP rather than an estrogenic effect. The epidemiological study on possibly reproductive effects in occupationally exposed women is inadequate for assessment of possible reproductive effects caused by DBP in humans in the working environment.

Based on the available reproduction, fertility and developmental studies and according to EC Criteria, dibutyl phthalate is placed in Category III for effects on fertility and in Category II for effects on developmental toxicity and is labelled with R-phrase 62: “Possible risk of impaired fertility” and R-phrase 61: “May cause harm to the unborn child”.

Annex 5 - further information on BBP

4.1.2.9 Fertility, development and endocrine activity.

BBP has been assessed for potential toxic effects on fertility, reproductive organs, development, and endocrine activity following exposure almost exclusively in rats. Only one developmental study in mouse and one *in vivo* study for estrogenic activity in mice was reported. Most of the available reproduction toxicity information results from oral exposure, however, the endocrine activity in rats and mice have also been evaluated after subcutaneous injection of BBP. A summary of the most important observations and a table of the critical effects of BBP with respect to fertility including effects on the reproductive organs, development and endocrine activity are included in the end of each section (**Table 4.27** and **Table 4.29**).

4.1.2.9.1 Fertility studies BBP, animals

In a new 2-generation study male and female CD (Sprague-Dawley) rats (40-45 days old), 30 animals/sex/dose (F0 generation) were administered Butyl Benzyl Phthalate (BBP) in the feed at doses of 0, 750, 3,750, and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day for 10 weeks (Tyl et al., 2004). Animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with exposure continuing. F0 and F1 males were necropsied after the delivery period, and a histopathologic evaluation was performed on 10 animals from the high dose group and the control group. The following organs were evaluated; pituitary, liver, thyroid gland, seminal vesicles with coagulating glands and fluids, epididymis with contents and fluids, prostate, testes and pancreas. An andrological assessment was also performed which included; reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenisation-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production. On the day of birth, post natal day (pnd) 0 anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were standardised to 10 pups (5/sex) on pnd 4. On pnd 11-13 all F1 male pups were examined for retained nipples/aerolae on the ventrum. At weaning on pnd 21, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. Any remaining F1 male pups not selected as parents or for necropsy, which exhibited retained nipples, were also necropsied. On pnd 21 F0 or F1 females were necropsied, and histopathology was performed on 10 animals from the high dose group and from the control group. The following organs were evaluated: ovaries, vagina, uterus with oviducts and cervix, pancreas, pituitary, thyroid gland and liver, and other tissues with gross lesions identified as being treatment related. Selected F1 weanlings 30/sex/dose were administered BBP in the diet for a 10 weeks prebreed exposure period. Acquisition of vaginal patency in females and preputial separation in males were assessed. Vaginal cytology for estrus cyclicity in F1 selected females was evaluated during the last three weeks of the prebreed exposure period, and they were mated for a two-week period as described above. F1 males were necropsied after the F2 litters, parental F1 females were necropsied with histopathology, as described above, and F2 weanlings, up to three/sex/litter were necropsied. For all surviving F0 and F1 parental animals the following organs were weighed at scheduled sacrifice: ovaries, uterus with oviducts and cervix, pituitary, adrenal glands, liver, thyroid gland, seminal vesicles, epididymis with contents and fluid, spleen, prostate, testes, brain, kidneys, and pancreas. Results F0 parental systemic toxicity; Males and females: at 750 mg/kg bw/day significantly increased absolute and relative liver weight and relative kidney weight. Histopathological lesions in the liver mostly graded as minimal, and more abundant in female rats. At 250 mg/kg bw/day significantly increased absolute (male) and absolute and relative (female) kidney weight was reported. In females, at 750

mg/kg bw/day a significantly decreased body weight from study day 0 to 70 and during gestation and lactation was reported. Results F0 parental reproductive toxicity; In males no reproductive effects were reported, since the exposure to BBP started after they had achieved puberty. In females at 750 mg/kg bw/day significantly reduced absolute and relative paired ovaries weight and uterus weight were reported. Results F1 offspring toxicity; At 750 mg/kg bw/day a significant decrease in pup body weight per litter on pnd 0, 4, 7, 14 and 21 and in the 250 mg/kg bw/day group at pnd 7. In male offspring AGD was significantly ($p < 0.001$) decreased in a dose-related pattern from 250 mg/kg bw/day (1.89 mm compared to controls at 2.06 mm) and at 750 mg/kg bw/day (1.7 mm compared to controls at 2.06 mm). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced. At 750 mg/kg bw/day significant increase ($p < 0.01$) in male pups with one or more nipples (19.23% compared to 0% in the control group), and in the number of nipples per male (0.72 compared to 0 in the controls). In the 750 mg/kg bw/day group a significanlty increase ($p < 0.001$) in the percentage of male pups with one or more areolae (32.31% compared to 2.63% in the controls), and in the number of areolae per male (1.29 compared to controls at 0.07, $p < 0.01$). At weanling necropsy in males at 750 mg/kg bw/day a significant decrease in terminal body weight, in absolute thymus weight, in absoulte and relative spleen and testis weight, and in absolute epididymis weight was reported. In postwean males F1 a significant delay in the acquisition of puberty in F1 males was seen, evident as delayed age at preputial separation (45.2 compared to 40.9 in controls), and in the adjusted age at preputial separation (45.4 compared to 41.0 in controls). At weanling necropsy in females a significant decrease in terminal body weight, in absolute thymus weight, in absolute and relative spleen weight, and in absolute ovaries and uterus weight was reported. In postwean females F1 a significant delay in the acquisition of puberty was seen, evident as a delay in vaginal patency at 750 mg/kg bw/day (34.1 compared to 31.4 in controls) and in adjusted age at vaginal patency (34.4 compared to 31.5 in controls). Results F1 systemic toxicity; males: at 750 mg/kg bw/day significantly decreased body weight during preebreed exposure and at necropsy, significantly increased relative liver weight, relative adrenal gland weight, absolute and relative pancreas weight and relative pituitary weight. At 250 mg/kg bw/day significantly increased absolute and relative liver, kidney and pancreas weight. Females: at 750 mg/kg bw/ day significantly decreased body weight at necropsy. Histopathological lesions graded as minimal were reported, and were more abundant in females. Results F1 reproductive toxicity; At 750 mg/kg bw/day significantly reduced mating (70.0 compared to 96.7 in controls) and fertility (81.0 compared to 100.0 in controls) indices in F1 parents to make F2 offspring. Males: at 750 mg/kg bw/day significantly reduced absolute paired testis weight (2.8585 g compared to controls 3.5980 g), paired epididymis weight (1.2076 g compared to controls 1.3507 g), prostate weight (0.5626 g compared to controls 0.7556 g) and seminal vesicle with coagulating gland weight (1.7515 compared to controls 2.1455). The number of rats with histopathological changes in testis and epididymis in the 750 mg/kg bw/day group was 23 and 15 compared to 3 and 2 in controls. Furthermore, the epididymal sperm concentration (649.51 mil/g compared to 825.59 mil/g in controls), the percentage of motile sperms (52.1 compared to 68.6 in controls), and the percentage progressively motile sperm (42.1 compared to 57.3 in controls) were significantly decreased at 750 mg/kg bw/day compared to controls. In the 750 mg/kg bw/day group a significant increase in the number of males with one or more reproductive tract malformations was reported (16 compared to 1 in controls), as well as in the percentage of males with one or more reproductive tract malformations (53.3 compared to 3.33 in controls). These included in the testis: abnormal, missing, reduced in size, and/or undescended, and in the epididymis missing (right, left or bilateral) or reduced in size (right, left or bilateral). Microscopic findings in the 750 mg/kg bw/day dose group included in the epididymis; aspermia (8/24) and chronic inflammation 4/24, in the prostate gland; chronic inflammation (13/30), and in the testis; atrophy seminiferous tubule (15/29) and dilatation duct rete testis (7/29). Furthermore, at 750 mg/kg bw/day the number of implants sites per litter (12.35 compared to 15.86 in controls),

number of total pups per litter and the average number of live pups per litter on pnd 0 (11.4 compared to 14.2 in controls) and on pnd 4 (10.9 compared to 14.0 in controls) was significantly reduced compared to control animals. In females the absolute and relative uterus weight was increased compared to control animals. Results F2 offspring toxicity; During lactation at 750 mg/kg bw/day significantly reduced number of total pups per litter and live pups per litter on pnd 0 compared to control animals was reported. Furthermore, the average pup body weight per litter on pnd 7 (14.52 g compared to 16.91 g in controls), pnd 14 (29.53 g compared to 33.87 g in controls) and pnd 21 (44.63 compared to 50.01 g in controls) was significantly reduced compared to control animals. A significantly ($p < 0.05$) reduced AGD was reported in males at 250 mg/kg bw/day (1.99 mm compared to 2.05 mm in controls) and at 750 mg/kg bw/day (1.77 mm compared to 2.05 mm in controls, $p < 0.001$). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced (1.99 mm at 250 mg/kg bw/day and 1.79 mm at 750 mg/kg bw/day compared to 2.04 in controls). No effect on AGD was reported in females. In males a significant increase in the percentage of male pups with one or more nipples (16.46 compared to 0 in the control group) and in the number of nipples per male (0.51 compared to 0 in controls), and in the number of areolae per male (3.14 compared to 0.05 in controls) was reported at 750 mg/kg bw/day. At weanling necropsy in males a significantly reduced terminal body weight (45.89 g compared to 51.78 in conrols), absolute thymus (0.2048 g compared to 0.2360 g in controls), absolute (0.1549 g compared to 0.2106 g in controls) and relative (0.3335 g compared to 0.4056 g in controls) spleen weight, and paired testis weight (0.1949 g compared to 0.2432 g in controls) was reported at 750 mg/kg bw/day. In the 750 mg/kg bw/day group a significant increase in gross lesions were reported. These included missing epididymis in twenty male weanlings (20/54) (full or caput or corpus), missing seminal vesicle or reduced size in 5 male weanlings (5/54), and one male in the 250 mg/kg bw/day group had a missing testis. In females weanling at necropsy a significant reduced terminal body weight, reduced absolute thymus and ovaries weight, and reduced absolute and relative spleen weight was reported at 750 mg/kg bw/day. At 250 mg/kg bw/day a significant increase in uterus weight was reported. F2 offspring was not evaluated as postweanlings. In this study the NOAEL for parental systemic toxicity is 250 mg/kg bw/day based on organ weight changes and histopathological lesions in the liver. The NOAEL for effects on the reproductive system in offspring is 50 mg/kg bw/day based on a dose-realted reduction in AGD in both F1 and F2 offspring from 250 mg/kg bw/day. This effect was still statistically significant at 250 and 750 mg/kg bw/day when the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate. The study was performed in compliance with Good Laboratory Practice, and the US EPA OPPTS Testing Guideline. The NOAEL value for fertility was 250 mg/kg bw/day based on statistically singificantly reduced mating and fertility indices in F1 parents to make F2 offspring.

A recent 2-generation study is available (Nagao et al., 2000). In this study Sprague-Dawley rats (8 week old) (25 male or female/group) were administered oral doses of 0, 20, 100 or 500 mg/kg bw/day BBP by gavage. F0 male rats were treated for 12 weeks prior to 2-week cohabitation, and until necropsy (confirmation of fertility by pairing). F0 female rats were treated for 2 weeks prior to cohabitation until necropsy (including gestation, delivery, and lactation through postpartum day 21). F1 animals were treated by oral gavage after weaning (postnatal day 22) until necropsy (confirmation of fertility by pairing). At 13 weeks of age mating was permitted. The F0 animals were observed for clinical signs daily during the study. In female F0 rats estrous cycling was evaluated. Furthermore, brain, hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, ovaries, uterus, thyroid gland, and pituitary gland were weighed. The levels of prolactin, luteinizing hormone (LH), FSH, thyroidstimulating hormone (TSH), triiodothyronine (T_3), thyroxine (T_4) and estradiol (E_2) were measured in serum. Histopathologic examination of ovaries, uterus, vagina, liver, kidneys, mammary glands, thyroid gland, parathyroid gland, pituitary gland, and adrenal

glands was performed in 10 dams from the 500 mg/kg bw/day dose group and the control group. F0 male rats were necropsied after confirmation of fertility by pairing with females. The brain hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, testes, epididymis, ventral prostate, seminal vesicles, thyroid gland, and pituitary gland were weighed. The number of sperm in the right cauda epididymis and the percentage of motile sperm was determined. The levels of testosterone, LH, FSH, TSH, T_3, and T_4 were measured in serum. Histopathologic examination of testes, epididymis, prostate, seminal vesicle with coagulating gland, parathyroid gland, liver, kidneys, mammary glands, thyroid gland, pituitary gland, and adrenal glands was performed in 10 males each from the 500 mg/kg bw/day dose group and the control group. Observations in F1 offspring included; Numbers of live and dead pups for each litter were recorded after post natal day (pnd) 0 to 21, and the viability from pnd 0 to 4 (preculling) and from pnd 4 (postculling) to pnd 21 in each litter. Anogenital distance (AGD) was determined for each pup on pnd 4, 7, 14, and 21. On pnd 4 litters were culled randomly to eight (4 pups/sex/litter). Two pups/sex/litter in each group were examined for the development of neural reflexes, and for physical development. Sexual maturation measured as vaginal opening for female offspring (beginning on pnd 28) and preputial separation for male offspring (beginning on pnd 35) was assessed (2/sex/litter). On pnd 22 offspring (2/sex/litter) were necropsied. The testes, epididymis, and seminal vesicle with prostate in males, and ovaries and uterus in females were weighed. The testes in 10 male weanlings and ovaries in 10 female weanlings from all groups, and the epididymis, ventral prostate, and seminal vesicle with coagulating gland in 10 male weanlings and the uterus in 10 female weanlings from the 500 mg/kg group and the control group were examined histologically. Levels of testosterone, LH, TSH, FSH, T_3 and T_4 in male weanlings and of prolactin, LH, FSH, TSH, T_3, T_4, and E_2 in female weanlings were determined. One male and one female offspring from each litter of each group was subjected to behvioral and functional tests. At 13 weeks of age mating was permitted by pairing on a 1:1 basis within the same treatment group. The same measurements described above for pregnancy, delivery, lactation, and the evaluation of histology of internal organs including reproductive tissues, sperm motions and counts, and serum hormone levels were performed in male and female offspring. F2 pups were necropsied on pnd 21.

The results from the two-generation study are as following; *In parent animals* (F0) a significant decrease in body weight gain was reported in males at 500 mg/kg/day compared to control males, although no decrease in food consumption was evident. In females no significant difference among groups in body weight and food consumption prior to mating or during pregnancy or lactation were reported. No dose-related changes were reported in estrous cyclicity, fertility and lactation. A dose-dependent increase in kidneys weight in rats of both sexes (significant at 100 and 500 mg/kg bw/day in females, and at 500 mg/kg bw/day in males), and an increase in liver weight in males (significant at 500 mg/kg bw/day), and a decrease in the weight of the ovaries in females (significant at 500 mg/kg bw/day) were reported compared to control animals. No macroscopic or microscopic changes were observed in the reproductive system of males or females. A decrease in serum testosterone, T_3 and T_4 levels (significant at 500 mg/kg bw/day), and an increase in FSH (significant from 100 mg/kg bw/day) were reported in males compared to control males. In females a significant increase in serum concentrations of prolactin, and a significant decrease in T_4 were reported at 500 mg/kg bw/day compared to control females. *Preweanling (F1);* The viability in percentage during pnd 0-4 was significantly decreased at 500 mg/kg bw/day (96.7% versus 100% in controls). Body weight of male and female offspring at birth in the 100 and 500 mg/kg bw/day dose group was significantly decreased compared to control animals (male offspring: 6.4 g at 100 mg/kg bw/day and 6.3 g at 500 mg/kg bw/day compared to 6.8 g in control offspring, female offspring: 6.0 g at 100 and 500 mg/kg bw/day compared to 6.4 g in control offspring), and the body weight at 500 mg/kg bw/day was lower throughout the study, however, the viability was not affected. In the 500-mg/kg bw/day group a significant decrease in AGD at birth was reported in male offspring, and an increase in AGD was reported in female offspring compared to control animals. A significant

decrease in testis and epididymis weight in males, and a significant decrease in ovary weight and increase in uterus weight in females was reported in the 500 mg/kg bw/day group compared to control animals. Furthermore, a significant decrease in FSH concentration in males at 500 mg/kg bw/day, and in TSH concentrations in males at 100 and 500 mg/kg bw/day were observed compared to control animals. In females the level of T_3 was significantly decreased in the 100 and 500 mg/kg bw/day dose group compared to control females. Histopathologic examination revealed a significant decrease in the numbers of spermatocytes in the seminiferous tubules in the 500 mg/kg bw/day group compared to control males. Cryptorchidism or hypospadia was not observed in any dose groups. In females no histopathologic abnormalities were considered to be related to BBP exposure. *Postweanling (F1);* Preputial separation for male offspring in the 500 mg/kg bw/day group was significantly delayed compared to control males at pnd 40, while vaginal opening for female offspring in this group was not affected. BBP did not affect the reproductive ability, including delivery and lactation at any dose levels, whereas a significant reduction in the absolute weights of the testis, epididymis, prostate, seminal vesicle and spleen were reported at 10 or 18 weeks of age, and a significant increase in relative weight of the thyroid gland, adrenal glands, and liver weights was reported in males at 500 mg/kg bw/day compared to control males. A significant increase in the relative weight of kidneys in the 100 and 500 mg/kg bw/day dose group was reported compared to control males. However, no significant organ weight changes were reported in females. A significant decrease in serum concentrations of testosterone, LH, and T_4 were reported in male offspring at 500 mg/kg bw/day compared to control males. Furthermore, in the 500 mg/kg bw/day dose group histopathologic examination revealed significant increases in the incidence of atrophy of the seminiferous tubules with a decreased number of germ cells, a significant increase in the incidence of interstitial edema, and a significant increase in the incidence of decreased number of sperm in the epididymis compared to control males. In females no adverse changes in the ovaries or uterus in the 500 mg/kg bw/day dose group were reported. As regards the behavioral function tests, the only effect observed related to BBP exposure was a significant increase in the spontaneous motor activity in females in the 500 mg/kg bw/day dose group compared to control females, however, no effect was reported in males. *Preweanling F2;* In this group no significant adverse effects related to BBP exposure were reported including pup weight, viability, and development. From this study no NOAEL value for effects on fertility could be derived. The NOAEL value for effects on the reproductive organs in males was 100 mg/kg bw/day. This NOAEL value is based on atrophy of the testis, epididymis, and seminal vesicle at 10 or 18 weeks of age, and reduced reproductive organ weights in the F1 generation at the next higher dose. The NOAEL value for effects on development was 20 mg/kg bw/day based on reduced body weight in male and female offspring at birth at 100 and 500 mg/kg bw/day

As part of a NTP BBP feeding carcinogenicity study in F344/N rats (NTP, 1997) a modified mating study were conducted. Groups of 15 male F344/N rats were given 0, 300, 2,800 or 25,000 ppm BBP in feed (corresponding to approximately 20, 200 or 2,200 mg/kg bw/day of BBP) for 10 weeks. After the ten weeks exposure period the animals recovered for 2 days. Then a 7-days mating period was started. After mating the male rats were necropsied. All rats survived to the end of the study. The final mean body weight of the 25,000 ppm group (226 g) was significantly lower than those of the controls (320 g). No clinical findings related to BBP exposure were noted. A few minor haematological changes occurred at 25,000 ppm. There was some evidence of a minimal anemia, and the platelet count was increased. The absolute and relative prostate gland (0.276 and 1.23) and testis weight (0.442 and 1.97) of the 25,000 ppm males were significantly less than those of the controls (prostate gland; absolute 0.609, relative 1.93, testis; absolute 1.497, relative 4.73). Degeneration of the seminiferous tubule epithelium was observed in all males at 25,000 ppm. A dose-dependent decrease in epididymal spermatozoa concentration was reported. At 300 ppm the epididymal spermatozoa concentration per gram epididymal tissue was $324.14 \cdot 10^6$, at 2,800 ppm $261.47 \cdot 10^6$ and at 25,000 ppm $0.57 \cdot 10^6$. The control value was $373.94 \cdot 10^6$. The decrease was

statistically significant according to the published report at 2,800 ppm ($P \leq 0.05$). However, in this study the days of allowed recovery [days between mating (detection of sperm plug) and counting of epididymal spermatozoa concentration (necropsy)] varied within animals and BBP dose. In the 2,800 ppm group a higher number of rats were shown to have a shorter recovery period compared to control animals. The epididymal spermatozoa concentration after mating increased almost back to normal in the control group after two days, whereas in the 300 and 2,800 ppm group it was almost back to normal after 4 or 5 days, however, this information is based on a limited number of observations. When days of recovery were taken into account in a covariate analysis of variance on the epididymal spermatozoa concentration from the control, 300 and 2,800 ppm group, the decrease in epididymal spermatozoa concentration was not statistically significant at 2,800 ppm at the 5% level ($p = 0.07$), however, a dose-dependent decrease was still evident (control; $382.5 \cdot 10^6$, 300 ppm; $340.7 \cdot 10^6$ and 2,800 ppm $282.2 \cdot 10^6$). Ten females mated to 25,000 ppm males were initially found to be sperm positive, none of these females were pregnant at necropsy. There were no significant differences in litter data between the controls and the 300 and 2,800 ppm groups. The NOAEL from this study based on reduced epididymal spermatozoa concentration was 300 ppm corresponding to 20 mg/kg bw/day of BBP. The NOAEL for fertility was 2,800 ppm corresponding to 200 mg/kg bw/day of BBP. This study was performed in compliance with GLP. A reproductive toxicity screening study according to OECD 421 Test Guideline was performed in rats. This study was performed to validate the OECD 421 Test Guideline protocol. BBP was chosen for this validation because BBP was known to have effects both on fertility parameters and on development on the conceptus (Agarwal et al., 1985; Hammond et al., 1987; NTP, 1989). The conclusion from the authors was that the OECD 421 Test Guideline scores BBP correctly as a reproductive toxicant. In the study RIVM-bred WU rats (10 males and 10 females/group) were exposed by gastric intubation to 250, 500 or 1,000 mg/kg/day of BBP. After dosing of both sexes for 14 days, males and females were paired (1:1), and allowed to mate for a maximum of 14 days, whilst dosing was continued. Males were dosed further daily, and killed and necropsied after a total dosage period of 29 days. Female rats were dosed until postpartum day 6, and then killed and necropsied. At 1,000 mg/kg effects were found on the body weight gain and food consumption in both male rats (63 g compared to control values at 80 g) and pregnant female rats (69 g compared to control values at 118), the pregnancy rate was reduced, testis and epididymis weights were significantly reduced (4.2 g compared to control values at 4.9 g), testicular degeneration accompanied by interstitial (Leydig) cell hyperplasia and appearance of cellular debris were increased as were time to conception and postimplantation loss. Corpora lutea, implants per dam, and pre-implantation loss were not different between controls and exposed groups. The number of live pups at day 1 and 6 after birth were lower in the 1,000 mg/kg/day group, 1.5 and 0.8 compared to control rats, 9.4 and 9.2. A reduced mean pup weight at day 1 was reported at 500 mg/kg/day of BBP (7.0 g in control animals and 6.5 g at 500 mg/kg/day), however, the pups were of similar weight compared to controls on post natal day 6. Furthermore, statistical significance was observed on a per pup basis as opposed to a litter basis which is more appropriate for these studies. No abnormalities were found in the offspring except for one pup in the low-dose group with a displaced digit of one paw. The NOAEL for effects on reproductive organs were 500 mg/kg bw/day. The NOEL for effects in offspring was 250 mg/kg bw/day of BBP based on reduced pup weight on postantal day 1 from 500 mg/kg bw/day (Piersma et al., 1995).

A one-generation reproduction study was performed in Wistar rats (Crl:WI (WU) BR) (male, 225 - 272 g 12/group, female, 185 - 266 g 24/group). BBP was administered at dietary concentrations of 0 (control), 0.2, 0.4 and 0.8% over one generation producing 2 litters. Clinical signs, body weight, food consumption, fertility and reproductive performance were evaluated, combined with microscopic examination of male and female reproductive organs (ovaries, uterus, vagina, testes,

epididymis, seminal vesicle, prostate, coagulating gland, pituitary) and the liver. No mortality or clinical signs reported, were considered to be caused by the treatment; one male and one female parent rat in the control group were killed during the study. Live birth index was 97, 98, 100 and 99% in the control, 0.2, 0.4 and 0.8% BBP group and viability index day 4-21 was 100, 97, 100 and 97% in the control, 0.2, 0.4 and 0.8% BBP group. Gross necropsy findings in parent rats or pups that died during lactation did not indicate the presence of any significant treatment related effects. A reduction in mean body weight was observed in the females in the 0.8% BBP group (263.3 ± 3.6) when compared to control animals (266.8 ± 3.5) during the gestational and lactational periods of the two litters. Reduced food consumption was reported at 0.8% BBP during both gestational and lactational periods, and was considered related to BBP exposure. A slight increase in absolute liver weights was found in the females of the 0.8% BBP group (8.71 and control 8.24) and in the males of the 0.4% (14.81) and 0.8% (14.98) BBP groups compared to control levels at (14.11). An increase in relative liver weight was only statistically significant for the females in the 0.8% BBP group (33.04 and control 30.88). Microscopic examination of the organs of the reproductive tract did not reveal any treatment related effects. On the basis of the results described above, the NOAEL for parental toxicity was 0.4% BBP in the diet based on effects on the liver (206 mg/kg bw/day for males, 217 mg/kg bw/day for females), and the NOAEL for reproductive performance and development of the offspring was 0.8% BBP in the diet, the highest dose tested (418 mg/kg bw/day for males, 446 mg/kg bw/day for females). The study was performed in compliance with GLP. The study was performed according to EEC Annex 5 Directive 79/831/EEC and OECD Guidelines No. 415 (Monsanto, 1993).

4.1.2.9.2 Effects on the reproductive organs, aimals

A 14-day dietary fertility study was conducted in adult, male Fisher 344 rats given levels of 0, 0.625, 1.25, 2.5 and 5% of BBP in the diet corresponding to approximately 0, 312, 625, 1,250 or 2,500 mg/kg bw/day of BBP. When expressed relative to body weight significant increases in liver and kidney weights were reported in all BBP groups (liver; 4.52, 5.19, 4.97, 4.46, compared to control value at 3.88, kidney; 0.809, 0.857, 0.840, 0.882, compared to control value at 0.763). A statistically significant reduction in total body, and absolute thymus, testis, epididymis, prostate and seminal vesicle weights were reported in the 2.5% and 5% BBP dose groups, whereas a statistically significant decrease in the relative organ weight was only reported in the thymus, testes, epididymis (only in the 5% group) and seminal vesicle at 2.5 and 5%. Histology revealed dose-dependent and statstistically significant atrophy of the testis, prostate and seminal vesicles at 2.5 and 5%, atrophy of the thymus and epididymis at 5%, and the presence of immature sperm cells in the tubular lumens and necrosis of the tubular epithelium in the caput epididymis at 2.5% (5/8 and 8/10 animals) and 5% BBP (3/9 and 10/10 animals). In the liver a mild multifocal chronic hepatitis was reported in the 5% BBP dose group, and in the kidney scattered cases of renal proximal tubular regeneration in a small number of animals in all dose groups were evident. Plasma testosterone concentration was significantly decreased in the 5% BBP group while follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were increased at 2.5% and 5.0% BBP. Since exposure to 5% BBP produced a generalised toxicosis this general toxicity may have caused or contributed to the organ-specific lesions observed at this dose. The NOAEL for effects on male reproductive organs was 1.25% corresponding to 625 mg/kg bw/day of BBP. The LOAEL for increases in relative liver and kidney weight was 0.625% of BBP in the diet corresponding to 312 mg/kg/day (Agarwal et al., 1985).

In a 28 days repeated dose toxicity study in young Cpb-WU male rats (28 days of age), rats were treated daily with BBP by gavage (Piersma et al., 2000). The study was a part of a developmental toxicity study reported under the section “Developmental studies, BBP”. The BBP doses used were

0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg bw/day, with three animals per group. Necropsy was performed one day after the last dosage. The liver, kidney, thymus, thyroid, spleen and testis were weighted and processed for histology. The results were as following: No changes in food consumption were reported after exposure to BBP. Body weight gain was decreased from 1,250 mg/kg bw/day, however, the decrese was not statistically significant. Relative liver weight was statistically significantly increased from 750 mg/kg bw/day (6.14, 6.45, 6.09, 6.66 and 6.64 compared to control value at 5.16). Liver PCoA as an index of peroxisome proliferation showed a similar response. A dose-dependent increased trend in relative kidney weight was reported from 750 mg/kg bw/day. However, the increase was not statistically significant. No statistically significant decrease in thymus and thyroid weight was reported, however, a trend towards a decrease was observed. No effects in spleen were reported. In testis a dose-related decrease was reported in relative testis weight from 750 mg/kg bw/day, however, the decrease was statistically significant first at 1,250 mg/kg bw/day (1.03 at 750 mg/kg/day, 0.74 at 970 mg/kg/day, 0.41 at 1,250 mg/kg/day, 0.28 at 1,600 mg/kg/day and 0.24 at 2,100 mg/kg/day compared to control value at 1.15). Histopathologic analysis of the testis revealed severe atrophy from 970 mg/kg bw/day. LH and FSH was increased at 1,250 mg/kg bw/day and a statistically significant decrease in testosterone level was reported from 450 mg/kg bw/day (1.7 at 450 mg/kg/day, 2.0 at 580 mg/kg/day, 3.8 at 750 mg/kg/day, 2.7 at 970 mg/kg/day, 1.8 at 1,250 mg/kg/day, 2.7 at 1,600 mg/kg/day, and 1.1 at 2,100 mg/kg/day compared to control value at 9.1). The study concluded a LOAEL of 970 mg/kg bw/day based on testis atrophy as the most critical reproductive effect in male rats in this study. The testicular atrophy was reported in the presence of a 20% increase in relative liver weight. In summary, the NOAEL for effects on the reproductive organs was 750 mg/kg bw/day. The NOAEL for systemic toxicity based on increased liver weight was 580 mg/kg bw/day.

In a 26 week dietary toxicity study (NTP Rapport No. 458, 1997), male Fisher F344/N rats (15/group) were given 0, 300, 2,800, 8,300 and 25,000 ppm BBP corresponding to 30, 180 and 550 or 1,660 mg/kg bw/day. The non-reproductive effects from the 26 week study are described in Section 4.1.2.6. The main effects on male sex organs and fertility are described in this section. Effects on fertility were studied at doses of 0, 2,800, 8,300 and 25,000 ppm BBP, and were only observed in the 25,000 ppm group. The pregnancy rate for females mated with 25,000 ppm dosed BBP males was 0/30. Epididymal spermatozoa concentrations at 0, 2,800, 8,300 and 25,000 ppm BBP were $284 \cdot 10^6$, $285 \cdot 10^6$, $376 \cdot 10^6$ and $2.06 \cdot 10^6$. In this study the control animals were shown to have an epididymal spermatozoa concentration lower than expected ($284 \cdot 10^6$ per gram cauda tissue). This phenomenon was explained in a study remark by the study director to may be due to an inadequate mincing of the cauda epididymis in these animals. Other control values for epididymal spermatozoa concentrations/gram epididymis from various studies are $365 \pm 107 \cdot 10^6$ in Fisher 344 rats (Balzak et al., 1985), $522 \cdot 10^6$ (Aajfes et al., 1980), $574.9 \pm 37.6 \cdot 10^6$ in Sprague-Dawley rats (NTP, 1991), $443 \pm 40 \cdot 10^6$ in Fisher 344 rats (van Birgelen et al., 1999), and $298.7 \pm 14.3 \cdot 10^6$ in Wistar rat (TNO, 1998). No information is given about mincing of the BBP treated tissue. The absolute right cauda, epididymis, and testis weight at 25,000 ppm was significant less than in control animals. The incidence of hypospermia and atrophy of the seminiferous tubule in the testis, and hypospermia in the epidydimis was significantly greater than in control animals. The degenerative changes of the testis and epididymis in the 25,000 ppm males were qualitatively and quantitatively similar to those observed in males in the 10-week study. The NOAEL for fertility and reduced epididymal spermatozoa concentration in this 26 weeks study was 8,300 ppm BBP corresponding to 550 mg/kg bw/day (NTP, 1997). This study was performed in compliance with GLP.

Several studies with BBP were conducted by Lake et al. (1978) and are reported in the following paragraphs. It should be noted that these studies are less comprehensive and detailed than the NTP studies.

Sprague-Dawley rats (6 animals/group) were treated daily by gastric intubation at dose levels of, 160, 480 or 1,600 mg/kg/day for 14 days. Control animals received corresponding quantities of corn oil vehicle. At 1,600 mg/kg/day a statistically significant increase in relative liver weight was reported (6.3 g compared to control value at 5.2 g). The administration of 1,600 mg/kg/day of BBP was found to cause a marked depression of both the absolute (0.82 compared to control value at 1.89) and relative testis weight (0.44 compared to control value at 0.99). At 1,600 mg/kg/day histopathologic examination revealed severe testicular atrophy in > 50% of the seminiferous tubules in all animals examined. Similar, but less severe lesions were observed by histopathologic examination in one of three animals examined from the 480 mg/kg/day group. However, the effect at 480 mg/kg/day was not statistically significant. The NOEL from this study was 160 mg/kg bw/day based on testicular atrophy reported in one of three animals at 480 mg/kg/day (Lake et al., 1978).

A similar test was performed with 480 and 1,600 mg/kg/day of BBP for 14 days in both Sprague-Dawley and Wistar rats. A significant depression in absolute and relative testis weight was only observed in rats receiving 1,600 mg/kg/day in Sprague-Dawley rats (0.68 and 0.34 compared to control levels at 2.03 and 0.95) and in Wistar rats (1.03 and 0.60 compared to control levels at 1.93 and 1.02). Histopathologic examination was performed on testis from all animals. 480 mg/kg/day did not induce any histopathologic changes in Wistar rats, however, in one Sprague-Dawley rat testicular atrophy < 25% was reported in one of six animals examined. In the 1,600 mg/kg/day group all animals from both strains were affected and the extent of the lesions being more severe in the Sprague-Dawley rats. For Wistar rats the NOAEL from this study was 480 mg/kg bw/day, and for Sprague-Dawley rats the LOAEL was 480 mg/kg bw/day (Lake et al., 1978).

In a 4-day study the testicular effects of BBP and the monoester derivatives of BBP were examined after oral administration. Administration of 1,600 but not 800 mg/kg/day of BBP was sufficient to reduce both the absolute (0.69 compared to control 0.87) and the relative (0.64 compared to control 0.76) testis weight of Sprague-Dawley rats within 4 days. Histopathologic examination revealed atrophic changes in three of six animals in the 800 mg/kg/day group and in five of six animals in the 1,600 mg/kg/day group. The severity of the atrophic lesions was enhanced at the highest dose level of BBP. Mono-n-butyl phthalate (MBuP) administered in doses equimolar to 1,200 mg/kg/day of the diester, caused a depression of both the absolute (0.96 compared to control 1.28) and relative (0.72 compared to control 0.95) testis weight, whereas monobenzyl phthalate (MBeP) only depressed the absolute testis weight (1.06 compared to control 1.28). Histopathologic examination of section of the testis from all animals treated with either MBuP or MBeP revealed atrophic changes. The effects of MBuP were more severe than those with MBeP (Lake et al., 1978).

In two 3 months subchronic studies in Sprague-Dawley and Wistar rats the animals were fed diets containing 2,500 to 20,000 ppm or 2,500 to 12,000 ppm BBP (corresponding to 188, 375, 750, 1,125, 1,500 or 151, 381, 960 mg/kg bw/day). In these studies no effects on testis were reported either as weight changes or histopathologically. However, in Sprague-Dawley rats an increase in the relative liver weight was reported at 1,125 mg/kg bw/day and higher, and in Wistar rats at 960 mg/kg bw/day. An increase in relative kidney weight was reported from 381 mg/kg bw/day in Wistar rats and histopathological changes in the pancreas from 381 mg/kg bw/day and in the liver at 960 mg/kg bw/day. For more detailed description of these studies see Section 4.1.2.6 (Hammond et al., 1987). In Wistar rats the NOAEL was 151 mg/kg bw/day based on increased kidney weight at

doses ≥ 381 mg/kg bw/day. In female Sprague-Dawley rats the NOAEL was 375 mg/kg bw/day based on liver and kidney weight increases at doses ≥ 750 mg/kg/day, and in males the NOAEL was 750 mg/kg/day based on liver weight increase at doses ≥ 1,125 mg/kg/day.

In a 4 week oral subacute toxicity study, Sprague-Dawley rats were administered BBP in food at doses from 500 to 4,000 mg/kg bw/day. Adverse reactions were reported in males from 2,000 to 4,000 mg/kg bw/day, and included testicular atrophy. Histopathological changes in the testis were reported in a dose-dependent manner from 1,500 mg/kg bw/day, whereas no changes were reported in the liver. The few high-dose animals that survived were allowed to recover for 4 weeks, and in these animals testicular atrophy was still evident in some animals. For more detailed description of the study see Section 4.1.2.6. The NOAEL for testicular atrophy was 1,000 mg/kg bw/day (Hammond et al., 1987).

4.1.2.9.3 Effects on the reproducitve organs, humans

Duty et al. (2003) studied whether the general population levels of phthalate monoesters in urine were associated with altered semen quality. The levels of mono-esters measured in urine reflected recent exposure to phthalates, since phthalates have short half-lives, and from all routes of exposure, oral, dermal, inhalation and ingestion. In this study male partners (168 men between 20 and 54 years of age) of sub-fertile couples were recruited. Semen parameters were dichotomized based on WHO (1999) reference values for sperm concentration (less than 20 million/ml) and motility (less than 50% motile) and Tygerberg Strict criteria for morphology (less than 4% normal). The comparison group was men with all three semen parameters above the reference values. Eight urinary phthalate monoesters were measured in a single spot urine sample collected on the same day as the semen sample [monoethyl phthalate (MEP), monomethyl phthalate (MMP), monoethylhexyl phthalate (MEHP), monobutyl phthalate (MBuP) monobenzyl phthalate (MBeP), monooctyl phthalate (MOP), monoisononyl phthalate (MINP), and monocyclohexyl phthalate (MCHP)]. The phthalate metabolites were measured with high performance liquid chromatography and tandem mass spectrometry. Specific gravity adjusted phthalate levels were dichotomized using median into high and low categories. The unadjusted median levels of urinary phthalate monoester concentrations in µg/L urine were; 156 for MEP, 10.3 for MBeP, 15.9 for MBuP, 5.7 for MEHP, and 7.5 MMP, reflecting exposure to diethyl phthalate, butyl benzyl phthalate, dibutyl phthalate, diethyl hexyl phthalate and dimethyl phthalate. These levels can be compared to phthalate monoester levels measured in Blount et al. (2000); Hoppin et al. (2002); NHANES, see Section 4.1.2.1. However, it has to be taken into account that in this study the levels of phthalate monoesters were only measured in males. The results from the Duty et al. (2003) study indicated that median monobutyl phthalate (MBuP) levels were associated with sperm motility and sperm concentration below the reference values with odds ratio (95% confidence interval) of 2.37 (1.13 to 5.00) and 2.41 (0.80 to 7.23) which means that they were 2.37 and 2.41 times more likley to have sperm motility or sperm concentrations below the reference value. The median mono benzyl phthalate (MBeP) levels were also associated with sperm motility, morphology, and sperm concentration below the reference values with odds ratio of 1.8 (0.9 to 3.9), 2.1 (0.9 to 5.1) and 2.7 (0.8 to 8.5). The authors concluded from the study that there were dose-response relations for MBuP and MBeP for one or more of the semen parameters studied, and suggestive evidence for MMP for sperm morphology. For the other monoesters, no clear correlations were found.

4.1.2.9.4 Summary fertility and effects on the reproductive organs, BBP

Reproductive effects of BBP and its major metabolites MBuP and MBeP in rats following oral administration both by gavage or in the diet have been investigated in studies of different duration (from 4 days to 26 weeks, and in 2-generation studies). The main effects reported include a decrease in the relative weight of testis, damage to the testis, epididymis, prostate, seminal vesicle and to reduced epididymal sperm concentrations, and at high BBP concentrations reduced fertility, in addition to increases in relative liver and kidney weights. The determined NOEL/NOAEL/LOAEL values form the various studies are given in **Table 4.27**.

Study Design	Effect Level	Critical Effect	Reference
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; Administration in feed; 0, 750, 3750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day of BBP.	NOAEL for fertility: 250 mg/kg bw/day of BBP based on reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. NOAEL for developmental effects: 50 mg/kg bw/day of BBP based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring	Fertility: reduced mating and fertility indices in F1 parents to make F2 offspring at 750 mg/kg bw/day. Development: reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day	Tyl et al. (2004)
Sprague-Dawley rats; two-generation study; 25/sex/group; Administration by gavage; 0, 20, 100 and 500 mg/kg bw/day BBP	NOAEL: 20 mg/kg bw/day BBP for developmental effects based on decreased body weight in offspring from 100 mg/kg bw/day. No NOAEL value could be derived for effects on fertility. NOAEL for effects on the reproductive organs: 100 mg/kg bw/day	Fo; decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovarie weight in females at 500 mg/kg/day. A decrease in testosteron (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day in F1 postweaning. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day as well. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	Nagao et al. (2000)

Study Design	Effect Level	Critical Effect	Reference
Fisher 344 rats male; 14 days; Administration in diet; 0.625, 1.25, 2.5 and 5% (312, 625, 1,250 and 2,500 mg/kg bw/day BBP)	NOAEL: 625 mg/kg bw/day BBP for effects on reproductive organs LOAEL: 312 mg/kg/day BBP for effects on liver and kidney	At doses ≥1,250 mg/kg bw/day body, thymus, testes, epididymis and prostate weight decrease, histopathologic changes in testes, prostate and seminal vesicle with the presence of immature sperm and necrosis in tubular epithelium, increased levels of LH and FSH. At 2,500 mg/kg bw/day decreased progesterone levels, general toxicosis.	Agrawal et al. (1985)
Cpb-WU male rats, 4 weeks of age; Administration of BBP by gavage 28 days; 3/group; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600, 2,100 mg/kg bw/day BBP	NOAEL for effects on the reproductive organs (reduced testis weight): 750 mg bw/kg/day BBP NOAEL for systemic toxicity (increased liver weight): 580 mg/kg bw/day	Liver weight increase from 750 mg/kg bw/day. A dose related decrease in testis weight from 750 mg/kg bw/day, however, statistically significant from 1,250 mg/kg bw/day. Decreased testosterone levels from 450 mg/kg bw/day. Severe testicular atrophy from 970 mg/kg bw/day.	Piersma et al. (2000)
Fisher 344 rats; 15/male/group; 10 weeks; Administration in diet; 300, 2,800 and 25,000 ppm (20, 200 and 2,200 mg/kg bw/day BBP)	NOAEL: 300 ppm (20 mg/kg bw/day BBP) for sperm effects. NOAEL: 2,800 ppm (200 mg/kg bw/day BBP) for fertility	At doses ≥ 200 mg/kg bw/day decreased epididymal spermatozoa concentration. At 2,200 mg/kg bw/day alterations in haematological values, decreased body, prostate and testes weight, degeneration in seminiferous tubules, no pregnancy after mating.	NTP (1997)
Fisher-344 male rats; 15 male/group; 26 weeks; Administration in diet; 0, 2,800, 8,300, and 25,000 ppm (0, 180, 550, 1,660 mg/kg bw/day BBP.)	NOAEL: 8,300 ppm (550 mg/kg bw/day BBP) for fertility and sperm effects.	Fertility; At 25,000 ppm decreased fertility, testis, epididymis weight, and epididymal spermatozoa conc. Degenerative changes in testis and epididymis. Other toxic effects see Table 4.24.	NTP (1997)
RIVM-bred WU-rats; 10/sex/group; 14 days prior to and throughout mating; gastric intubation; 250, 500 and 1,000 mg/kg bw/day BBP.	NOEL: 250 mg/kg bw/day BBP based on reduced pup weight at 500 mg/kg bw/day; NOAEL 500 mg/kg bw/day for effects on reproductive organs	At 1,000 mg/kg bw/day decreased body weight, pregnancy rate, live pups, pup weight, and epididymis weight, testicular degeneration. At 500 mg/kg bw/day slighty reduced pup weight.	Piersma et al. (1995)
Wistar rats; Administration in diet over one generation producing two litters; 0.2, 0.4 and 0.8% BBP	NOAEL parental: 0.4% (206 mg/kg bw/day BBP male and 217 mg/kg/day BBP female) based on increased liver and kidney weight NOAEL reproductive performence and developmental effects: 0.8% (418 mg/kg bw/day BBP male and 446 mg/kg bw/day BBP female). Based on reduced reproductive performance.	At 0.8% reduced body weight gain and food intake in dams. Slight increase in absolute and relative liver weight in female.	Monsanto (1993)

Study Design	Effect Level	Critical Effect	Reference
Sprague-Dawley rats; 6 male/group; 14 days; gastric intubation; 160, 480 and 1,600 mg/kg bw/day BBP.	NOEL: 160 mg/kg bw/day BBP	At 480 mg/kg bw/day histopathologic changes in testis in one of three rats examined, at 1,600 mg/kg bw/day decreased testes weight with testicular atrophy.	Lake et al. (1978)
Wistar rats and Sprague-Dawley rats; 6/male/group; 14 days; gastric intubation; 480 and 1,600 mg/kg bw/day BBP.	NOAEL Wistar rat: 480 mg/kg bw/day BBP LOAEL Sprague-Dawley rats: 480 mg/kg bw/day BBP	At 480 mg/kg bw/day testicular atrophy in one Sprague-Dawley rat. At 1,600 mg/kg bw/day decreased testes weight with testicular atrophy in all rats. Sprague-Dawley rats were more severely affected than Wistar rats.	Lake et al. (1978)
Sprague-Dawley rats; 6/male/group; 4 days; gastric intubation; 800 and 1,600 mg/kg bw/day of BBP, 855 mg/kg bw/day of MBuP and 985 mg/kg bw/day of MBeP		At doses ≥ 800 (BBP), 855 (MBuP) and 985 MBeP) mg/kg bw/day reduced testes weight and testicular atrophy.	Lake et al. (1978)
Sprague-Dawley rats; 5-10/sex/group; 4 weeks; Administration in diet; 500, 1,000, 1,500 2,000, 3,000 and 4,000 mg/kg bw/day BBP.	NOAEL: 1,000 mg/kg bw/day BBP	At doses ≥ 1,500 mg/kg bw/day body weight decrease. From 1,500 mg/kg bw/day testicular atrophy. From 2,000 mg/kg/day stiffness while walking and bleeding around nares.	Hammond et al. (1987)
Sprague-Dawley rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 20,000 ppm (corresp. to approx. 188, 375, 750, 1,125, 1,500 mg/kg bw/day BBP)	NOAEL female: 375 mg/kg bw/day BBP NOAEL male: 750 mg/kg bw/day BBP	At doses ≥ 750 mg/kg bw/day kidney and liver weight increase in females, at doses ≥ 1,125 mg/kg bw/day liver weight increase in males.	Hammond et al. (1987)
Wistar rats; 10/sex/group; 3 month; Administration in diet; 2,500 – 12,000 ppm (corresp. to approx. 151, 381, 960 mg/kg bw/day BBP)	NOAEL male and female: 151 mg/kg bw/day BBP	At doses ≥ 381 mg/kg bw/day kidney weight increase, urinary pH decrease. At 960 mg/kg bw/day body weight decrease, liver weight increase, slight anaemia, and histopathologic changes in liver and pancreas.	Hammond et al. (1987)

As regards the effects on fertility or reproductive organs following administration of BBP to rats in the diet (Tyl et al., 2004; Agarwal et al., 1975; NTP, 1997; Hammond et al., 1987) or via gavage (Piersma et al., 1995; Piersma et al., 2000; Lake et al., 1978; Nagao et al., 2000), reduced mating and fertility indices, decreases in testis weight, histopathological changes in testis, and hormonal changes have been reported. These effects have in the majority of the studies been reported at BBP doses equal to (Hammond et al., 1987, 4-week diet study) or higher than those which have induced other effects, such as variations in absolute and relative weights of the liver and kidney and histopathological changes such as atrophy in the liver and pycnotic nuclei, acinar atrophy and slight fibrosis in the pancreas. Exceptions includes, when BBP is administered by gavage, a 14 days and a 4 days study in Sprague-Dawley rats (Lake et al., 1978), and a 28 days study in Cpb-WU rats (Piersma et al., 2000). In the Lake 14 days study, minimal testicular atrophy was reported in one of three animals examined at 480 mg/kg bw/day (Lake et al., 1978). In the 4 days study atrophic changes in the testis in 3 of 6 animals at 800 mg/kg bw/day of BBP were reported. In the 28 days study by Piersma et al. (2000) a decrease in testosterone level was reported from 450 mg/kg/day. Exceptions include, when BBP is administered in the diet, a 10 week fertility study (NTP, 1997). In this study a dose-related decrease in epididymal spermatozoa concentration compared to control animals was reported from 200 mg/kg bw/day (2,800 ppm) ($p \leq 0.05$) of BBP and the NOAEL from this study was 20 mg/kg bw/day of BBP. When taking into account days of recovery in males in the 10 week NTP study (days from positive sperm plug to necropsy) in a covariate analysis of variance, on the epididymal spermatozoa concentration, the decrease in epididymal spermatozoa concentration at 2,800 ppm was not statistically significant at the 5% level compared to control animals ($p = 0.07$). However, the dose-dependent decrease in epididymal spermatozoa

concentration was still evident. In the parallel 26-week oral toxicity study in rats where BBP was administered in the diet (NTP, 1997) the control value for epididymal spermatozoa concentration may not be valid due to a reported possible inadequate mincing of the cauda epididymis tissue from control animals. The NOAEL for reduced epididymal spermatozoa concentration and fertility in the 26 week study was 550 mg/kg bw/day. In a new 2-generation study (Tyl et al., 2004) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations was reported (53.33% compared to 0% in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study. In another recent two-generation study (Nagao et al., 2000) increased serum FSH in F0 males was reported from 100 mg/kg bw/day, and at 500 mg/kg bw/day a decreased testosterone level. In F1 males (18 weeks old) a decrease in testis, epididymis and ventral prostate weight, a decrease in testosterone and LH levels, and athrophy of the seminiferous tubules with decreased number of germ cells, and a decreased number of sperm in the epididymis were reported, accompanied with reduced body weight and an increased relative liver and kidney weight. No effect on fertility was reported in this study at any dose levels (20, 100 or 500 mg/kg bw/day). From the Nagao (2000) study no NOAEL value could be derived for effects on fertility. The NOAEL value for effects on the reproductive organs in males was 100 mg/kg bw/day, based on weight changes and atrophy of the reproductive organs in the F1 generation at 10 or 18 weeks of age at 500 mg/kg bw/day. The NOAEL value for developmental effects was 20 mg/kg bw/day based on reduced body weight in male and female F1 offspring from 100 mg/kg bw/day.

Only one human study is available where the relation between exposure to phthalates and semen quality was evaluated. In this study an association was found between high levels of mono butyl phthalate and/or mono benzyl phthalate in the urine and altered semen quality including semen concentration, semen motality and semen morphology (Duty et al., 2003). Due to the mixed exposure to various phthalates it is difficult to conclude that the effect observed on semen quality is related only to BBP exposure. Furthermore, the phthalates were only measured in a single spot urine sample in a relative small group of men (168) derived from subfertile couples.

The National Toxicology Program (NTP) Center for the evaluation of risk to human reproduction has used a phthalate expert panel to evaluated the reproductive and developmental toxicity of BBP and other phthalates. This expert panel has concluded that the database on reproductive toxicity is suffucient to judge that oral exposure to BBP can cause reproductive toxicity in rats (Kavlock et al., 2002).When taking the available data base into account a NOAEL value at 100 mg/kg bw/day for effect on the reproducitve organs is considered to be used in the risk characterisation from the study of Nagao et al. (2000). This NOAEL value is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day.

In the above summarised studies, effects on male reproductive organs and/or fertility are reported after administration of BBP in doses equal to or higher than those which induce minimal systemic toxicity such as relative organ weight changes, and in some studies histopathological changes in the liver and pancreas. Furthermore, since signs of testicular toxicity, evident as a dose-dependent decrease in epididymal spermatozoa concentration and atrophy of the testis, and decreased testosterone and FSH levels, are reported in the absence of effects in other organs, BBP may affect fertility. Based on the available data BBP is proposed classified with Xn R62 (Repro. Cat. 3) “Possible risk of impaired fertility”, according to EU criteria.

4.1.2.9.5 Developmental studies, BBP, animals

Developmental toxicity of BBP was studied in Swiss DC-1 mice. Timed-pregnant mice received BBP (> 96% purity) in feed 0 (control), 0.1, 0.5, 1.25 and 2.0% corresponding to 0, 182, 910, 2,330 and 4,121 mg/kg bw/day from gestation day (gd) 6 to 15 (major organogenesis). Dams (27-30/group) were sacrificed on gd 17. No maternal or embryo/foetal effects were reported at 0.1% BBP (182 mg/kg bw/day). At 0.5% BBP (910 mg/kg bw/day), maternal effects were limited to a reduction (15%) in dam weight gain (gd 6-15), however no reduction was reported in adjusted weight gain; prenatal mortality per litter (15% versus 8% for controls) and malformed foetuses per litter (14% versus 4% for controls) were significantly increased. At 1.25% BBP (2,330 mg/kg bw/day) dam weight gain was reduced by 66% (gd 6-15) and 25% (gd 0-17 corrected for uterine weight). Absolute liver weight was decreased and relative liver and kidney weights were increased in the absence of treatment-related microscopic lesions; foetal weight per litter was 83% of controls; prenatal mortality per litter (93% versus 8% for controls) and malformed foetuses per litter (89% versus 4% for controls) were increased. The 2.0% BBP group (4,121 mg/kg bw/day) was eliminated after evaluation of 14 dams since all conceptuses were resorbed. The NOAEL for maternal and developmental toxicity, according to the author was 0.1% BBP (182 mg/kg bw/day). 0.5% BBP (910 mg/kg bw/day) is by the authors described to produce minimal evidence of maternal effects (15% reduction in maternal body weight gain during treatment), and significant developmental toxicity, evident as foetal death per litter (15% versus 8% for controls) and percent malformed foetuses per litter (14% versus 4% for controls) (NTP, 1990).

Developmental toxicity of BBP was studied in Sprague-Dawley rats. Time-pregnant rats received BBP (~96% purity) in feed 0 (control), 0.1, 0.5, 1.25 and 2.0% corresponding to 0, 419, 1,102 and 1,641 mg/kg bw/day from gestation day (gd) 6-15 (major organogenesis). Dams (27-30/group) were sacrificed on gd 20. At 1.25% BBP (1102 mg/kg bw/day), dam weight gain (gd 6-15) was reduced by 37% and relative liver weight was increased. The percent foetuses with variations/litter were increased (41.03% versus 19.04% for controls). The percent malformed foetuses per litter (5.9% versus 2% for controls) were increased. At 2% BBP (1,641 mg/kg bw/day), dam weight gains (gd 6-15 and gd 0-20 corrected for uterine weight) were reduced by 93% and 17%; foetal weight was 80% of controls; resorptions per litter (40% versus 4% for controls) and malformations per litter (53% versus 2% for controls) were increased. Maternal food/water intake was unchanged or increased, except for reduced food intake at 2% BBP (gd 6-9) in rats. The NOAEL for maternal and developmental toxicity was 0.5% BBP (419 mg/kg bw/day) (NTP, 1989).

Developmental toxicity of BBP was investigated in a recent study in pregnant and non-pregnant Cpb-WU rats (8 weeks of age) (Piersma et al., 2000). Pregnant rats received BBP from gd 6-15 (short time exposure) or gd 6-20 (long time exposure) by gavage. The study was performed to investigate developmental toxicity following both the classic exposure duration from gd 6-15 or from gd 6-20, the last which is considered to be a more sensitive exposure duration used for the study of developmental effects in animals. Ten dose groups of BBP were studied for both exposure durations, 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg bw/day. Ten pregnant rats per group for 270, 350, 580, 970, 1,600 or 2,100 mg/kg bw/day and 25 animals per group for 450, 750 or 1,250 mg/kg bw/day. To each dose group 3 nonmated females were added for comparison with pregnant female rats exposed to BBP. The nonpregnant rats were exposed to BBP for 10 or 15 days. Body weight and food consumption was determined on gd 0, 6, 11, 16 and 21. Necropsy was performed on gd 21. Before termination blood was taken for haematological and biochemical analysis which included serum ALAT (alanin aminotransferase), ASAT (aspergine aminotransferase), testosterone, progesterone, LH, FSH, PRL (prolactin) and estradiol. Liver protein and PCoA activity (palmitoyl CoA oxidase) was also assessed as an index for peroxisome

proliferation. After termination the weight of liver kidneys, thymus, thyroid and spleen were determined and a liver lobe was isolated for biochemical analysis. A histological examination was performed on the respective organs. Corpus Lutea, implantation sites, early and late resorptions and foetuses with early and late death were counted, and external anomalies of living foetuses were registered. Odd foetuses were processed for skeletal staining and examination, even foetuses were fixed for morphological analysis according to Barrow and Taylor (1969). The results in pregnant rats and non-pregnant rats were as following:

Maternal effects

At 1,600 and 2,100 mg/kg bw/day several animals died during the first five days of exposure due to toxicity of BBP. Two animals in the 1,600 mg/kg bw/day group died at dosing on day 10. Decreased food consumption was observed from 1,250 mg/kg bw/day during the first 5 days of dosing. Afterwards there was no difference in food consumption between the dose groups. Pregnant rats showed a statistically significant dose-related reduction in body weight gain from 750 mg/kg bw/day. The reduction was more pronounced in the long exposure group than in the short. In non-pregnant rats no dose-related differences in body weight gain were reported. A statistically significant dose-related increase in relative liver weight was reported from 750 mg/kg bw/day in the short time exposure group, and from 580 mg/kg bw/day in the long time exposure group. Similar observations were reported in the non-pregnant rats. Haematological biochemical analysis of ALAT, ASAT, PCoA showed increases from 750, 970 and 270 mg/kg bw/day after long time exposure, whereas, only minor increases were reported after short time exposure. Peroxisome proliferation was reported only at the three highest doses of BBP. Effects on kidneys of pregnant rats included a statistically significant increase in kidney weight from 750 mg/kg bw/day in both exposure groups, and from 970 mg/kg bw/day in the non-pregnant long time exposure group. Effects on thymus, thyroid and spleen were reported at doses from 970, 1,250 and 750 mg/kg bw/day in pregnant rats. In non-pregnant rats only an effect on spleen weight was reported at 2,100 mg/kg bw/day. Haeamatologic evaluation revealed an increase in haematocrit from 750 mg/kg bw/day in pregnant rats, whereas no effects were reported in nonpregnant rats. Endocrinology showed a statistically significant reduction in progesterone levels in pregnant rats from 270 mg/kg bw/day in the long time exposure group, and from 1,250 mg/kg bw/day in the short time exposure group, indicating that the effects at lower doses may be reversible, as short time exposure ended 6 days before necropsy. It was concluded from the study that the LOAEL for maternal toxicity was 580 mg/kg bw/day based on a dose-related liver weight increase, and the corresponding NOAEL 450 mg/kg bw/day.

Developmental effects

No effects on Corpora Lutea were reported. Early resorption was increased from 1,600 mg/kg bw/day, whereas late resorption was increased from 750 mg/kg bw/day in both exposure groups. Foetal weights were statistically significantly decreased from 450 mg/kg bw/day after short time exposure and from 350 mg/kg bw/day after long time exposure. From 750 mg/kg bw/day skeletal anomalies were reported to increase. A low incidence of foetal ovary malformations was found from 580 mg/kg bw/day, mainly after long time exposure. Rather than the spheric shape in unaffected animals, these ovaries had an elongated shape reminiscent of an earlier developmental stage, suggesting a retardation of morphologic development. The incidence of retarded foetal testicular caudal migration showed a dose-related increase from 580 mg/kg bw/day, the incidence being higher after long time exposure as compared to short time exposure to BBP. A decrease in relative foetal testis weight was only reported after long time exposure in a doserelated way from 270 mg/kg bw/day. At 270 mg/kg bw/day a statistically significant reduction was reported (0.282 compared to control value at 0.337). At the two next higher doses 350 and 450 mg/kg bw/day, the

foetal testis weight was 0.315 and 0.305. This reduction was not statistically significant, however, from 580 mg/kg bw/day the reduction in foetal testis weight was statistically significant. It was concluded from the study that the most sensitive foetotoxic effect of BBP was a relative foetal testicular weight reduction with a LOAEL at 270 mg/kg bw/day. The morphological effects on testis and ovary at 580 mg/kg bw/day and higher were testicular dislocation and ovary malformation. In summary, the maternal NOAEL was 450 mg/kg bw/day based on statistically significant incresed liver weight at 580 mg/kg/bw/day. The LOAEL based on a dose-related reduction (statistically significant at 270, 580, 750 mg/kg bw/day and further, but not at 350 and 450 mg/kg bw/day) in relative foetal testis weight was 270 mg/kg bw/day. The NOAEL for reduced foetal weight was 270 mg/kg bw/day.

The embryotoxic effects of butyl benzyl phthalate (BBP) and its two main metabolites mono-*n*-butyl (MBuP) and mono-*n*-benzyl phthalate (MBeP) were studied in 0F1 mice and Sprague-Dawley rats, *in vivo* and in whole embryo cultures (Saillenfait et al., 2003). *In vivo*, pregnant mice (15-23/group) and pregnant rats (7-13/group) received a single oral dose of BBP, MBuP or MBeP on gestaion day (gd) 8 and 10. The concentrations of BBP, MBuP and MBeP tested were 0, 0.9, 1.8, 3.6 and /or 5.4 mmol/kg, corresponding to 0, 280, 560, 1,120 and 1,690 mg/kg BBP; 0, 200, 400, 800, 1,200 mg/kg MBuP and 230, 460, 920 and 1,380 mg/kg MBeP. The foetuses were examined externally on gd 18 (mice) and 21 (rats). Results mice *in vivo*: Maternal deaths occurred within 24 hours after administration of 1,120 (1) and 1,690 (3) mg/kg BBP, 800 mg/kg (1) MBuP, and 920 (2) and 1,380 (5) mg/kg MBeP. BBP caused a statistically significant decrease in body weight gain on gd 9-18 in the 1,120 and 1,690 mg/kg dose group (13.2 ± 1.5 and 10.5 ± 1.3 compared to 22.9 ± 1.3 in controls). MBuP caused a statistically significant decrease in body weight gain on gd 9-18 in the 400, 800 and 1,200 mg/kg dose group (13.4 ± 1.4, 7.6 ± 1.4 and 7.8 ± 1.0 compared to 22.7 ± 1.4 in controls). MBeP caused no statistically significant reduction in body weight gain on gd 9-18. No changes were reported in the corrected body weight gain (body weight gain on gd 0-18 minus gravid uterus weight) following exposure to BBP, MBuP and MBeP. In the two highest BBP dose groups a statistically significant reduction in live foetus/litter was reported and in the three highest dose groups of MBuP a statistically significant reduction in live foetus/litter was reported. No statistically significant effect on live foetus/litter was reported following exposure to MBeP. A statistically significant increase in the percentage of resorptions/litter was reported in the three highest dose groups of BBP and MBuP, and in the highest dose group of MBeP. A dose-depended increase in malformed foetuses per litter was reported from 560 mg/kg BBP, 200 mg/kg MBuP and 920 mg/kg MBeP. The mean foetal weight/litter was statistically significantly decreased only at the highest doses of BBP and MBuP. Results rats *in vivo*: Maternal lethality occurred after administration of 1,690 (1) mg/kg BBP, and 920 (1) and 1,380 (5) mg/kg MBeP. No statistically significant decrease in body weight gain, or corrected body weight gain was reported following exposure to BBP, MBuP and MBeP. No effects on live foetus/litter and percentage of post-implantation loss/litter were reported following exposure to BBP, MBuP and MBeP. A slight increase in malformed foetuses per litter was reported from 1,120 mg/kg BBP, and only at 460 mg/kg MBeP. Results *in vitro* mice: gd 8 mouse embryos were cultured for 46 hours in the presence of MBuP and MBeP at the following concentrations 0.5, 1, 2 and 5 mM. Exposure to MBuP resulted in concentration-related effects on growth, development and morphology. All parameters assessed (yolk sac diameter, crown-rump length, head length, number of somites and morphological score) were significantly different from the controls at 5mM MBuP. MBeP induced significantly reductions in the crown-rump length, head length and number of somites at concentrations ≥ 1 mM, and in the yolk sac diameter and developmental score at concentrations ≥ 2 mM. Results *in vitro* rats: gd 10 rat embryos were cultured for 46 hours in the presence of MBeP at the following concentrations 0.5, 1, 2 and 3 mM. MBeP induced concentration related effects on growth and development. There was a slight but statistically significant reduction in head length and

morphological score from 1 mM. All parameters assessed (see above) were significantly lower than the control from 2 mM (by 16-31%). These data indicate that the cultured mouse embryos did not appear intrinsically more sensitive to MBuP and MBeP, than the rat embryos. The authors suggested that the species sensitivity observed *in vivo* after oral administration of BBP, MBuP or MBeP during early organogenesis, might be due to maternal factors i.e. toxicity and/or kinetics.

In a developmental toxicity study performed by Gray and coworkers (Gray et al., 2000) pregnant Sprague-Dawley rats (5/group, block 1) were gavaged daily with 750 mg/kg bw/day of BBP or corn oil from gestation day 14 through postnatal day (pnd) 3. A second block with the same dosing regime was conducted to repeat the positive effects reported in the first block. Eight Sprague-Dawley rats/group were used in block 2. In block 1 the animals were necropsyed at 3-4 month of age, and in block 2 at 4-7 monthes of age. Reproductive organ weights were taken from almost every male from each litter (45 male pups/11 litters versus 77 male pups/19 litters in controls in block 1 and 30 male pups/10 litters versus 45 male pups/17 litters in block 2). No reduction in maternal weight gain was reported during gestation and up to pnd 3. The mean pup weight on a per litter basis was statistically significant reduced at birth (5.78 g compared to 6.84 g in the control group). In male offspring treated with BBP a reduced anogenital distance (about 30%), and reduced paired testis weight (about 35%) at day 2 of age was reported on a per litter basis, furthermore, significant decreases in seminal vesicle (1,154 versus 1,857 in controls), ventral prostate (398 versus 685 in controls), paired epididymis (966 versus 1,293 in controls), cauda epididymis (182 versus 312 in controls) were reported on a per litter basis. As infants, 70% of males displayed femalelike areolas/nipples at day 13 of age compared to 0% in the control group on a per litter basis. At necropsy, malformations in the androgen-dependent organs and testis were reported in 84% of male offspring treated with BBP on a per litter bais. Hypospadias were reported in 29% of the male offspring on a per litter basis. Di(2-ethylhexyl) phthalate (DEHP) was also tested in this study, and the results demonstrated that exposure to 750 mg/kg bw/day BBP or DEHP from gestation day 14 through pnd 3 severely altered sexual differentiation in the male rat with about equal potency, and that BBP altered male rat sexual differentiation in an antiandrogenic fashion. The developmental effects of BBP were also studied by Parks et al. (1999). In this study pregnant Sprague-Dawley rats were dosed by gavage with 750 mg/kg bw/day BBP from gestational day 14 to pnd 3. On pnd 2 anogenital distance (AGD), testes weight and *in vitro* testosterone production were measured. Testes weight and AGD was decreased for BBP exposed male pups, and the incidence of areolas on pnd 13 was increased. Testosterone production was not reduced by BBP treament. The authors describe that these antiandrogenic-like effects may results from reduced androgen production in the fetal Leydig cells and suggests that the testis is the target organ directly affected by perinatal BBP exposure. However, it remains to be determined whether these effects are mediated via direct action of BBP on fetal Leydig cells or through alterations of Sertoli cell paracrine secretions.

In a new 2-generation study male and female CD (Sprague-Dawley) rats (40-45 days old), 30 animals/sex/dose (F0 generation) were administered Butyl Benzyl Phthalate (BBP) in the feed at doses of 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day for 10 weeks (Tyl et al., 2004). Animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with exposure continuing. F0 and F1 males were necropsied after the delivery period, and a histopathologic evaluation was performed on 10 animals from the high dose group and the control group. The following organs were evaluated; pituitary, liver, thyroid gland, seminal vesicles with coagulating glands and fluids, epididymis with contents and fluids, prostate, testes and pancreas. An andrological assessment was also performed which included; reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenisation-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production. On the day of birth, post natal day (pnd) 0 anogenital distance (AGD)

was measured and body weights recorded for all live F1 pups in all litters. F1 litters were standardised to 10 pups (5/sex) on pnd 4. On pnd 11-13 all F1 male pups were examined for retained nipples/aerolae on the ventrum. At weaning on pnd 21, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. Any remaining F1 male pups not selected as parents or for necropsy, which exhibited retained nipples, were also necropsied. On pnd 21 F0 or F1 females were necropsied, and histopathology was performed on 10 animals from the high dose group and from the control group. The following organs were evaluated ovaries, vagina, uterus with oviducts and cervix, pancreas, pituitary, thyroid gland and liver, and other tissues with gross lesions identified as being treatment related. Selected F1 weanlings 30/sex/dose were administered BBP in the diet for a 10 weeks prebreed exposure period. Acquisition of vaginal patency in females and preputial separation in males were assessed. Vaginal cytology for estrus cyclicity in F1 selected females was evaluated during the last three weeks of the prebreed exposure period, and they were mated for a two-week period as described above. F1 males were necropsied after the F2 litters, parental F1 females were necropsied with histopathology, as described above, and F2 weanlings, up to three/sex/litter were necropsied. For all surviving F0 and F1 parental animals the following organs were weighed at scheduled sacrifice: ovaries, uterus with oviducts and cervix, pituitary, adrenal glands, liver, thyroid gland, seminal vesicles, epididymis with contents and fluid, spleen, prostate, testes, brain, kidneys, and pancreas. Results F0 parental systemic toxicity; Males and females: at 750 mg/kg bw/day significantly increased absolute and relative liver weight, and relative kidney weight. Histopathological lesions in the liver mostly graded as minimal, and more abundant in female rats. At 250 mg/kg bw/day a significantly increased absolute (male) and absolute and relative (female) kidney weight was reported. In females, at 750 mg/kg bw/day significantly decreased body weight from study day 0 to 70, and during gestation and lactation was reported. Results F0 parental reproductive toxicity; In males no reproductive effects were reported, since the exposure to BBP started after they had achieved puberty. In females at 750 mg/kg bw/day significantly reduced absolute and relative paired ovaries weight and uterus weight were reported. Results F1 offspring toxicity; At 750 mg/kg bw/day a significant decrease in pup body weight per litter on pnd 0, 4, 7, 14 and 21, and in the 250 mg/kg bw/day group at pnd 7. In male offspring AGD was significantly ($p < 0.001$) decreased in a dose-related pattern from 250 mg/kg bw/day (1.89 mm compared to controls at 2.06 mm) and at 750 mg/kg bw/day (1.7 mm compared to controls at 2.06 mm). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced. At 750 mg/kg bw/day significant increase ($p < 0.01$) in male pups with one or more nipples (19.23% compared to 0% in the control group), and in the number of nipples per male (0.72 compared to 0 in the controls). In the 750 mg/kg bw/day group a significanlty increase ($p < 0.001$) in the percentage of male pups with one or more areolae (32.31% compared to 2.63% in the controls), and in the number of areolae per male (1.29 compared to controls at 0.07, $p < 0.01$). At weanling necropsy in males at 750 mg/kg bw/day a significant decrease in terminal body weight, in absolute thymus weight, in absoulte and relative spleen and testis weight, and in absolute epididymis weight was reported. In postwean males F1 a significant delay in the acquisition of puberty in F1 males was seen, evident as delayed age at preputial separation (45.2 compared to 40.9 in controls), an in the adjusted age at preputial separation (45.4 compared to 41.0 in controls). At weanling necropsy in females a significant decrease in terminal body weight, in absolute thymus weight, in absolute and relative spleen weight, and in absolute ovaries and uterus weight was reported. In postwean females F1 a significant delay in the acquisition of puberty was seen, evident as a delay in vaginal patency at 750 mg/kg bw/day (34.1 compared to 31.4 in controls) and in adjusted age at vaginal patency (34.4 compared to 31.5 in controls). Results F1 systemic toxicity; Males: at 750 mg/kg bw/day significantly deceased body weight during preebreed exposure and at necropsy, significantly increased relative liver weight, relative adrenal gland weight, absolute and relative pancreas weight and relative pituitary weight. At 250 mg/kg bw/day significantly increased absolute and relative

liver, kidney and pancreas weight. Females: at 750 mg/kg bw/day significantly decreased body weight at necropsy. Histopathological lesions graded as minimal were reported, and were more abundant in females. Results F1 reproductive toxicity; At 750 mg/kg bw/day significantly reduced mating (70.0 compared to 96.7 in controls) and fertility (81.0 compared to 100.0 in controls) indices in F1 parents to make F2 offspring. Males: at 750 mg/kg bw/day significantly reduced absolute paired testis weight (2.8585 g compared to controls 3.5980 g), paired epididymis weight (1.2076 g compared to controls 1.3507 g), prostate weight (0.5626 g compared to controls 0.7556 g) and seminal vesicle with coagulating gland weight (1.7515 compared to controls 2.1455). The number of rats with histopathological changes in testis and epididymis in the 750 mg/kg bw/day group was 23 and 15 compared to 3 and 2 in controls. Furthermore, the epididymal sperm concentration (649.51 mil/g compared to 825.59 mil/g in controls), the percentage motile sperm (52.1 compared to 68.6 in controls), and the percentage progressively motile sperm (42.1 compared to 57.3 in controls) was significantly decreased at 750 mg/kg bw/day compared to controls. In the 750 mg/kg bw/day group a significant increase in the number of males with one or more reproductive tract malformations were reported (16 compared to 1 in controls), and in the percent males with one or more reproductive tract malformations (53.3 compared to 3.33 in controls). These included in the testis abnormal, missing, reduced in size, and/or undescended, and in the epididymis missing (right, left or bilateral) or reduced in size (right, left or bilateral). Microscopic findings in the 750 mg/kg bw/day dose group included in the epididymis; aspermia (8/24) and chronic inflammation 4/24, in the prostate gland; chronic inflammation (13/30), and in the testis; atrophy seminiferous tubule (15/29) and dilatation duct rete testis (7/29). Furthermore, at 750 mg/kg bw/day the number of implants sites per litter (12.35 compared to 15.86 in controls), number of total pups per litter and the average number of live pups per litter on pnd 0 (11.4 compared to 14.2 in controls) and on pnd 4 (10.9 compared to 14.0 in controls) was significantly reduced compared to control animals. In females the absolute and relative uterus weight was increased compared to control animals. Results F2 offspring toxicity; During lactation at 750 mg/kg bw/day significantly reduced number of total pups per litter and live pups per litter on pnd 0 compared to control animals was reported. Furthermore, the average pup body weight per litter on pnd 7 (14.52 g compared to 16.91 g in controls), pnd 14 (29.53 g compared to 33.87 g in controls) and pnd 21 (44.63 compared to 50.01 g in controls) was significantly reduced compared to control animals. A significantly ($p < 0.05$) reduced AGD was reported in males at 250 mg/kg bw/day (1.99 mm compared to 2.05 mm in controls) and at 750 mg/kg bw/day (1.77 mm compared to 2.05 mm in controls, $p < 0.001$). When the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values at 250 and 750 mg/kg bw/day were still significantly reduced (1.99 mm at 250 mg/kg bw/day and 1.79 mm at 750 mg/kg bw/day compared to 2.04 in controls). No effect on AGD was reported in females. In males a significant increase in the percentage male pups with one or more nipples (16.46 compared to 0 in the control group) and in the number of nipples per male (0.51 compared to 0 in controls), and in the number of areolae per male (3.14 compared to 0.05 in controls) was reported at 750 mg/kg bw/day. At weanling necropsy in males a significantly reduced terminal body weight (45.89 g compared to 51.78 in conrols), absolute thymus (0.2048 g compared to 0.2360 g in controls), absolute (0.1549 g compared to 0.2106 g in controls) and relative (0.3335 g compared to 0.4056 g in controls) spleen weight, and paired testis weight (0.1949 g compared to 0.2432 g in controls) was reported at 750 mg/kg bw/day. In the 750 mg/kg bw/day group a significant increase in gross lesions were reported. These included missing epididymis in twenty male weanlings (20/54) (full or caput or corpus), missing seminal vesicle or reduced size in 5 male weanlings (5/54), and one male in the 250 mg/kg bw/day group had a missing testis. In females weanling at necropsy a significant reduced terminal body weight, reduced absolute thymus and ovaries weight, and reduced absolute and relative spleen weight was reported at 750 mg/kg bw/day. At 250 mg/kg bw/day a significant increase in uterus weight was reported. F2 offspring was not evaluated as postweanlings. In this study the NOAEL for parental systemic toxicity is 250 mg/kg bw/day based on organ weight changes and histopathological lesions

in the liver. The NOAEL for effects on the reproductive system in offspring is 50 mg/kg bw/day based on a dose-realted reduction in AGD in both F1 and F2 offspring from 250 mg/kg bw/day. This effect was still statistically significant at 250 and 750 mg/kg bw/day when the male AGD was statistically analysed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate. The study was performed in compliance with Good Laboratory Practice, and the US. EPA OPPTS Testing Guideline.

A recent 2-generation study is available (Nagao et al., 2000). In this study Sprague-Dawley rats (8 week old) (25 male or female/group) were administered oral doses of 0, 20, 100 or 500 mg/kg bw/day BBP by gavage. F0 male rats were treated for 12 weeks prior to 2-week cohabitation, and until necropsy (confirmation of fertility by pairing). F0 female rats were treated for 2 weeks prior to cohabitation until necropsy (including gestation, delivery, and lactation through postpartum day 21). F1 animals were treated by oral gavage after weaning (postnatal day 22) until necropsy (confirmation of fertility by pairing). At 13 weeks of age mating was permitted. The F0 animals were observed for clinical signs daily during the study. In female F0 rats estrous cycling was evaluated. Furthermore, brain, hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, ovaries, uterus, thyroid gland, and pituitary gland were weighed. The levels of prolactin, luteinizing hormone (LH), FSH, thyroidstimulating hormone (TSH), triiodothyronine (T_3), thyroxine (T_4) and estradiol (E_2) were measured in serum. Histopathologic examination of ovaries, uterus, vagina, liver, kidneys, mammary glands, thyroid gland, parathyroid gland, pituitary gland, and adrenal glands was performed in 10 dams from the 500 mg/kg bw/day dose group and the control group. F0 male rats were necropsied after confirmation of fertility by pairing with females. The brain hearth, lung, liver, spleen, kidneys, adrenal glands, thymus, testes, epididymis, ventral prostate, seminal vesicles, thyroid gland, and pituitary gland were weighed. The number of sperm in the right cauda epididymis and the percentage of motile sperm were determined. The levels of testosterone, LH, FSH, TSH, T_3, and T_4 were measured in serum. Histopathologic examination of testes, epididymis, prostate, seminal vesicle with coagulating gland, parathyroid gland, liver, kidneys, mammary glands, thyroid gland, pituitary gland, and adrenal glands was performed in 10 males each from the 500 mg/kg bw/day dose group and the control group. Observations in F1 offspring included; Numbers of live and dead pups for each litter were recorded after post natal day (pnd) 0 to 21 and the viability from pnd 0 to 4 (preculling) and from pnd 4 (postculling) to pnd 21 in each litter. Anogenital distance (AGD) was determined for each pup on pnd 4, 7, 14 and 21. On pnd 4 litters were culled randomly to eight (4 pups/sex/litter). Two pups/sex/litter in each group were examined for the development of neural reflexes, and for physical development. Sexual maturation measured as vaginal opening for female offspring (beginning on pnd 28) and preputial separation for male offspring (beginning on pnd 35) was assessed (2/sex/litter). On pnd 22 offspring (2/sex/litter) were necropsied. The testes, epididymis, and seminal vesicle with prostate in males, and ovaries and uterus in females were weighed. The testes in 10 male weanlings and ovaries in 10 female weanlings from all groups, and the epididymis, ventral prostate, and seminal vesicle with coagulating gland in 10 male weanlings and the uterus in 10 female weanlings from the 500 mg/kg group and the control group were examined histologically. Levels of testosterone, LH, TSH, FSH, T_3 and T_4 in male weanlings and of prolactin, LH, FSH, TSH, T_3, T_4, and E_2 in female weanlings were determined. One male and one female offspring from each litter of each group was subjected to behvioral and functional tests. At 13 weeks of age mating was permitted by pairing on a 1:1 basis within the same treatment group. The same measurements described above for pregnancy, delivery, lactation, and the evaluation of histology of internal organs including reproductive tissues, sperm motions and counts, and serum hormone levels were performed in male and female offspring. F2 pups were necropsied on pnd 21.

The results from the two-generation study

In parent animals (F0) a significant decrease in body weight gain was reported in males at 500 mg/kg/day compared to control males, although no decrease in food consumption was evident. In females no significant difference among groups in body weight and food consumption prior to mating or during pregnancy or lactation was reported. No dose-related changes were reported in estrous cyclicity, fertility and lactation. A dose-dependent increase in kidneys weight in rats of both sexes (significant at 100 and 500 mg/kg bw/day in females, and at 500 mg/kg bw/day in males), and an increase in liver weight in males (significant at 500 mg/kg bw/day), and a decrease in the weight of the ovaries in females (significant at 500 mg/kg bw/day) were reported compared to control animals. No macroscopic or microscopic changes were observed in the reproductive system of males or females. A decrease in serum testosterone, T_3 and T_4 levels (significant at 500 mg/kg bw/day), and an increase in FSH (significant from 100 mg/kg bw/day) were reported in males compared to control males. In females a significant increase in serum concentrations of prolactin, and a significant decrease in T_4 were reported at 500 mg/kg bw/day compared to control females. *Preweanling (F1);* The viability in percentage during pnd 0-4 was significantly decreased at 500 mg/kg bw/day (96.7% versus 100% in controls). Body weight of male and female offspring at birth in the 100 and 500 mg/kg bw/day dose group was significantly decreased compared to control animals (male offspring: 6.4 g at 100 mg/kg bw/day and 6.3 g at 500 mg/kg bw/day compared to 6.8 g in control offspring, female offspring: 6.0 g at 100 and 500 mg/kg bw/day compared to 6.4 g in control offspring), and the body weight at 500 mg/kg bw/day was lower throughout the study, however, the viability was not affected. In the 500 mg/kg bw/day group a significant decrease in AGD at birth was reported in male offspring, and an increase in AGD was reported in female offspring compared to control animals. In this study the AGD was not adjusted for individual body weights. Only the absolute AGD was measured. A significant decrease in testis and epididymis weight in males, and a significant decrease in ovary weight and increase in uterus weight in females was reported in the 500 mg/kg bw/day group compared to control animals. Furthermore, a significant decrease in FSH concentration in males at 500 mg/kg bw/day, and in TSH concentrations in males at 100 and 500 mg/kg bw/day were observed compared to control animals. In females the level of T_3 was significantly decreased in the 100 and 500 mg/kg bw/day dose group compared to control females. Histopathologic examination revealed a significant decrease in the numbers of spermatocytes in the seminiferous tubules in the 500 mg/kg bw/day group compared to control males. Cryptorchidism or hypospadia was not observed in any dose groups. In females no histopathologic abnormalities were considered to be related to BBP exposure. *Postweanling (F1);* Preputial separation for male offspring in the 500 mg/kg bw/day group was significantly delayed compared to control males, while vaginal opening for female offspring in this group was not affected. BBP did not affect the reproductive ability, including delivery and lactation at any dose levels, whereas a significant reduction in the absolute weights of the testis, epididymis, prostate, seminal vesicle and spleen were reported, and a significant increase in relative weight of the thyroid gland, adrenal glands, and liver weights were reported in males at 500 mg/kg bw/day compared to control males. A significant increase in the relative weight of kidneys in the 100 and 500 mg/kg bw/day dose group was reported compared to control males. However, no significant organ weight changes were reported in females. A significant decrease in serum concentrations of testosterone, LH, and T_4 were reported in male offspring at 500 mg/kg bw/day compared to control males. Furthermore, in the 500 mg/kg bw/day dose group histopathologic examination revealed significant increases in the incidence of atrophy of the seminiferous tubules with a decreased number of germ cells, a significant increase in the incidence of interstitial edema, and a significant increase in the incidence of decreased number of sperm in the epididymis compared to control males. In females no adverse changes in the ovaries or uterus in the 500 mg/kg bw/day dose group were reported. As regards the behavioral function tests, the only effect observed related to BBP exposure was a significant increase in the spontaneous motor activity in females in the 500 mg/kg bw/day dose group compared to control females, however, no effect was reported in males. *Preweanling F2;* In this group no significant adverse effects related to BBP exposure were reported including pup

weight, viability, and development. From this study no NOAEL value for effects on fertility could be derived. The NOAEL value for effcts on development was 20 mg/kg bw/day based on reduced body weight in male and female offspring at birth at 100 and 500 mg/kg bw/day.

Several studies of the developmental toxicity of BBP and the major BBP metabolites (MBeP and MBuP) have been performed by Ema and coworkers (1990, 1991, 1992a, 1992b, 1992c, 1993a, 1994a, 1995a, 1995b, 1996 and 1998a; 2002) in Wistar rats after oral exposure. The following studies are described below.

The developmental toxicity of BBP was evaluated in pregnant rats exposed to various concentrations of BBP from day 0 of gestation to day 20, and killed on day 20 of pregnancy. The rats were exposed to 0.25, 0.5, 1.0 or 2.0% BBP in the diet corresponding to approximately 185, 375, 654 or 974 mg/kg bw/day (Ema et al., 1990) or to 2% BBP corresponding to 974 mg/kg bw/day (Ema et al., 1991). The number of pregnant rats/group was 13 to 17. Reduced maternal weight gain during pregnancy was observed at 375, 654 and 974 mg/kg bw/day. However, a reduced adjusted weight gain was only observed at 654 and 974 mg/kg bw/day. Embryotoxic effects were observed in the 0.5% group with a significant reduction in the number of live fetuses per litter (11.3 ± 3.8 versus 13.9 ± 1.6 in controls). In the 1.0% group a significantly reduced body weight of male and female foetuses, and in the 2.0% group complete resorption of all the implanted embryos were reported. Morphological examination of the foetuses revealed no evidence of teratogenesis. The maternal NOAEL from the Ema et al. (1990) study was 375 mg/kg bw/day based on a reduced adjusted weight gain from 654 mg/kg bw/day, and the NOAEL for offspring was 185 mg/kg bw/day based on reduced number of live fetuses per litter at 375 mg/kg bw/day. A pair-feeding study was performed in which the pregnant pair-fed rats received the same amount of diet consumed by the 2% BBP-treated pregnant rats. The pair-fed and 2% BBP-treated rats showed the same reductions in the adjusted weight gain. Higher incidence of post implantation losses were reported in pair-fed rats compared to control rats, however, the complete resorption of all the implanted embryos was not found in any of the pair-fed rats. It was concluded that the embryolethality in the 2% BBP exposed rats could be attributable to the effects of dietary BBP and not from reduced food consumption during pregnancy (Ema et al., 1991).

In another study the teratogenic potential of BBP was investigated in pregnant Wistar rats (10 pregnant rats/group). The rats were given BBP in corn oil once daily by gastric intubation (0, 500, 750 or 1,000 mg/kg bw/day) throughout the period of major organogenesis i.e. on days 7-15 of pregnancy. The rats were killed on day 20 of pregnancy. At 500 mg/kg bw/day reduced food consumption in the dams were reported, and in the 750 mg/kg bw/day group reduced body weight gain. However, a reduced adjusted body weight gain was only reported at 1,000 mg/kg bw/day. High maternal lethality was observed in the 1,000 mg/kg bw/day group. Complete resorption of all implanted embryos was observed in 3 of 10 dams at 750 mg/kg bw/day and in all of the 6 pregnant rats in the 1,000 mg/kg bw/day groups. At 750 mg/kg bw/day a significant decrease in foetal weight, and a decrease in the number of live fetuses per litter was reported, and a significant increase in the incidence of foetal malformations per litter. These malformations included external malformations, skeletal malformations (fusion of sternebrae), and internal malformations (dilatation of renal pelvis). The LOAEL maternal and NOAEL offspring from this study was 500 mg/kg bw/day (Ema et al., 1992a).

To determine if periods of exposure during pregnancy would modify the developmental toxicity of BBP, pregnant Wistar rats were given BBP by oral exposure at a concentration of 2% in the diet (974 mg/kg bw/day). The exposure period in the first (Ema et al., 1992b) study was on days 0-20, days 0-7 (the pre-implantation and pre-organogenesis period), days 7-16 (the organogenesis period)

or days 16-20 (the foetal period) of pregnancy and from day 0-20, 0-11 and 11-20 in the second (Ema et al., 1992c) study. The number of pregnant rats/group was 11 in both studies. In both studies, a pair-fed group of rats was included which received the same amount of feed as the feed intake of rats fed a diet containing 2% BBP. The pregnant rats were killed on day 20 of pregnancy in both studies. Pronounced effects on maternal body weight gain, adjusted body weight gain and food consumption during pregnancy were found regardless of the days on which BBP was given. No effect on preimplantation loss per litter was reported on exposure day 0-20, 0-7 or 0-11. A statistically significant increase in postimplantation loss per litter was reported after exposure in the early phase of pregnancy (day 0-20, 0-7, 0-11, and 7-16) as compared to control animals and pair fed rats, whereas no increase was found after exposure in the late phase of pregnancy (day 11-20 or 16-20). After exposure on pregnancy day 11-20 or 16-20 the incidence of teratogenic effects (cleft palate and fusion of the sternebrae) in the foetuses were significantly and markedly higher than in the control and pair-fed groups. The authors concluded that the teratogenic effect of BBP after oral administration during the organogenic period is primarily the result of the effect of exposure to BBP.

The teratogenic phase specificity of BBP during gestation on developmental toxicity was examined by a shorter duration of treatment. Pregnant Wistar rats were dosed once daily by gastric intubation with BBP dissolved in olive oil at a dose of 600, 750 or 1,000 mg/kg bw on days 7-9, 10-12 or 13-15 of pregnancy. Control rats received olive oil only, on the corresponding days. The pregnant rats were killed on day 20 of pregnancy (10 litters/group were examined). No information was provided regarding the evaluation of maternal effects. After exposure on pregnancy day 7-9 post-implantation the loss/litter and the number of dead foetuses per litter was significantly increased and the number of live foetuses per litter and body weight of live foetuses was significantly decreased at 750 and 1,000 mg/kg bw/day of BBP. After exposure on pregnancy day 10-12 postimplantation loss and the number of dead foetuses per litter was significantly increased at 750 and 1,000 mg/kg bw/day of BBP and number of litters totally resorbed was significantly increased at 1,000 mg/kg bw/day of BBP. Number of live foetuses per litter, and body weight of live foetuses were significantly decreased at 1,000 mg/kg bw/day of BBP. After exposure on pregnancy day 13-15, post-implantation loss and the number of dead foetuses per litter was significantly increased at 750 and 1,000 mg/kg bw/day of BBP, and number of live foetuses per litter was significantly decreased at 750 and 1,000 mg/kg bw/day of BBP. However, different patterns of malformations were induced during the different exposure periods. These included malformations [external malformations (cleft palate), skeletal malformations (fusion and/or absence of cervical vertebral arches or thoracic vertebral arches)] after exposure to 750 or 1,000 mg/kg bw/day of BBP on pregnancy day 7-9 or 13-15, whereas no malformations were reported after exposure on pregnancy day 10-12. The sex ratio of live foetuses was comparable across all groups, except for the group treated on days 10-12 with 1,000 mg/kg bw/day. No significant effects in any reproductive parameter were found in pregnant rats given a dose of 600 mg/kg bw/day. The authors concluded also from this study that the susceptibility to the teratogenic effect of BBP varies with the developmental stage at the time of BBP administration. The NOAEL for offspring from this study was 600 mg/kg bw/day (Ema et al., 1993a).

In the Ema et al. (1994a) study, the embryolethality of BBP during early pregnancy was investigated by studying the effect of BBP on the uterine and ovarian weight and plasma progesterone levels. The rats were exposed to BBP (2%) on day 0-7, 0-9 and 0-11, and the rats were sacrificed on day 7, 9 and 11 of pregnancy (6 pregnant rats/group). No effect on pre-implantation the loss/litter was reported on exposure day 0-7, 0-9 or 0-11. A marked increase in post-implantation loss per litter was reported on exposure day 0-11, only due to the method of quantifying post implantation loss (the presence of a heartbeat on day 11 of pregnancy).

Furthermore, compared to control animals a significant decrease in the uterine and ovarian weight and plasma progesterone levels in all groups except for the ovarian weight on day 7 were reported. No significant differences in pre- and post-implantation loss, uterine weight, and plasma progesterone levels were reported between the control and pair fed rats. The postimplantation embryonic loss due to BBP exposure during early pregnancy therefore, may seem to be mediated via the reduction in plasma progesterone levels, and impairment of luteal function.

To further elucidate the effect of BBP on early embryolethality this effect of BBP was investigated in pregnant (10-14/group, 218-240 g) and pseudopregnant (11-13/group, 216-263 g) Wistar rats. Decidualized pseudopregnant rats were used to study the direct effect of BBP on maternal reproductive physiology. Rats were given BBP by gastric intubation at 0, 250, 500, 750 or 1,000 mg/kg bw/day on days 0-8 of pregnancy and the pregnancy outcome was determined on day 20 of pregnancy. The same doses of BBP were given to pseudopregnant rats, and on day 9 of pseudopregnancy the rats were sacrificed and blood samples were collected for progesterone measurements, and the uterus and ovaries were weighted and served as an index for uterine decidualisation. *Results pregnant rats*; Maternal; at 1,000 mg/kg bw/day of BBP two deaths were reported. A significant decrease in body weight gain was observed on day 0-9 in all BBP-treated groups, with a full recovery on day 9-20, except for the 1,000 mg/kg bw/day group. A significant reduction in food consumption was also reported in all exposed groups on day 0 – 9, however, no differences were reported on day 9 – 20. No effects were seen in the adjusted body weight gain between BBP-treated animals and control animals, possible due to recovery of maternal animals from gd 9-20. The female rats in the BBP-treated groups at higher doses showed a reddish staining of the facial fur and/or piloerection. *Foetal effects*; BBP caused a significant increase in the incidence of pre-implantation loss per litter at 1,000 mg/kg bw/day, and of postimplantation loss per litter at 750 and 1,000 mg/kg bw/day. A significant decrease in body weight of live foetus was observed at 500, 750 and 1,000 mg/kg bw/day. *Results pseudopregnant rats:* At 1,000 mg/kg bw/day two deaths were reported. A significant decrease in body weight gain was reported on day 0-9 at 500, 750 and 1,000 mg/kg bw/day together with a decreased food intake. A significant lower ovarian weight was reported in the 750 and 1,000 mg/kg bw/day group. No differences in the number of corpora lutea were reported in the BBP treated groups compared to the control group. A dose-dependent decrease in uterine weight (statistically significant at 750 and 1,000 mg/kg bw/day) and a trend towards decreased serum progesterone levels at doses ≥ 500 mg/kg bw/day were reported. The results in the pregnant and pseudopregnant rats may indicate a correlation between suppression of the responsiveness of the uterus and the early embryonic loss after administration of relatively high doses of BBP. The LOAEL maternal was 250 mg/kg bw/day, NOAEL offspring was 250 mg/kg bw/day and NOAEL pseudopregnant rats was 250 mg/kg bw/day in this study (Ema et al., 1998a).

The effects of butyl benzyl phthalate (BBP) on the development of the reproductive system in male offspring were studied in Wistar rats (Ema et al., 2002). In this study pregnant rats (16/group) were given BBP by gastric intubation at doses of 250, 500 or 1,000 mg/kg on days 15 to 17 of pregnancy. No death was found in any groups. Maternal effects included a statistically significant decrease in maternal body weight gain at 500 mg/kg (23 ± 6) and 1,000 mg/kg (23 ± 9) compared to 31 ± 5 in the control group. However, no effect was found on the adjusted maternal body weight gain (maternal weight gain excluding the gravide uterus). A statistically significant decrease in maternal food consumption was reported at 500 mg/kg (34 ± 6) and at 1,000 mg/kg (33 ± 6) compared to 45 ± 6 in the control group. A statistically significant decrease in the number of live foetuses per litter was found at 1,000 mg/kg (12.6 ± 1.9 compared to 14.6 ± 1.5 in the control group). The weight of the male and female foetuses was significantly decreased at 1,000 mg/kg (male 3.82 ± 0.65 compared to 4.58 ± 0.32 in controls, female 3.67 ± 0.56 compared to 4.27 ± 00.31 in controls). A

significant increase in the incidece of foetuses/litter with undescended testes was found at 500 (54/14) and 1,000 (97/16) mg/kg compared to 0/16 in the control group. Furthermore, a statistically significant decrease in the anogenital distance (AGD) of male foetuses was observed at 500 and 1,000 mg/kg. The AGD/cube root of body weight ratio in male foetuses was also significantly reduced from 500 mg/kg. The AGD/cube root of body weight ratio in female foetuses in the BBP treated groups were comparable to those in the control group. It was concluded by the authors of the study that BBP given to pregnant rats during gestation day 15-17 produced adverse effects on the development of the reproductive system in male offspring. The NOAEL for maternal toxicity was 250 mg/kg BBP and the NOAEL for developmental toxicity 250 mg/kg.

The effect of BBP on development, and on maternal and embryonic zinc metabolism was studied in pregnant rats (Uriu-Adams et al., 2001), since different chemicals may induce the synthesis of maternal metallothionein (Mt) in the rat. Metallothionein can bind zinc, which may lead to a reduction in the transfer of zinc to the embryo (Taubeneck et al., 1994; Daston et al., 1991) and thus, embryonic zinc deficiency-induced abnormal development of the conceptuses. In this study female Wistar rats (180-200 g, 9-16 rats/group) were administered BBP diluted in corn oil by gavage at doses of 0, 250, 1,000, 1,500 or 2,000 mg/kg bw/day once daily on gestation day (gd) 11, 12 and 13. The animals were killed on gd 20. At doses ≥ 1,000 mg/kg bw/day signs of marked maternal toxicity were reported including diarrhoea, blood in faeces, bloody discharge from the nose and eyes, and reduced activity. In addition, two deaths were reported at 2,000 mg/kg bw/day. The body weight on gd 20 was significant lower in the 2,000 mg/kg bw/day group. Food intake was lowest in the 2,000 mg/kg bw bw/day group from gd 12 to 16 compared to the other BBP dosed groups and the control group, however, food intake was similar in all groups from gd 17 to 19. No effects on maternal haematocrits, liver or kidney weights were observed. Increased placental weights were seen at doses ≥ 1,500 mg/kg bw/day of BBP. At 2,000 mg/kg bw/day some of the placentas had light green patches. BBP affected reproductive outcome in a dose-dependent manner. Reduced foetal weights were observed at doses ≥ 1,500 mg/kg bw/day and at 2,000 mg/kg bw/day fewer live foetuses and higher percentages of resorptions were seen in the litters compared to the other groups. Gross anomalies were reported in the pups at 1,000, 1,500 and 2,000 mg/kg bw/day upon visual inspections. Effects of BBP on skeletal ossification on gd 20 were significantly changed at 1,500 and 2,000 mg/kg bw/day. A higher incidence of skeletal anomalies (rudimentary ribs and cleft palate) were reported from 1,000 mg/kg bw/day compared to the control and 250 mg/kg bw/day group. Maternal liver Mt was determined in all dose groups. A dose dependent tendency towards increased concentration of maternal liver Mt was seen (0.78 ± 0.13, 0.63 ± 0.11, 0.89 ± 0.15, 1.67 ± 0.23, 2.82 ± 0.76 in the control group, 250, 1,000, 1,500 and 2,000 mg/kg bw/day), however, not statistically significant. At 2,000 mg/kg bw/day of BBP two dams had a six fold higher liver Mt concentration compared to controls, and in these two dams 93-100% resorptions were reported. Maternal plasma concentrations of Zn was not statistically significantly increased among the exposure groups (11.88 ± 0.77, 11.38 ± 0.67, 12.88 ± 0.62, 11.25 ± 0.71 and 14.46 ± 1.29 in the control group, 250, 1,000, 1,500 and 2,000 mg/kg bw/day exposure groups). It was concluded from the study that BBP was not a strong inducer of Mt, and that the teratogenicity of BBP does not appear to be due to alterations in maternal and/or embryonic Zn metabolism (Uriu-Adams et al., 2001).

Sharpe and coworkers (1995) studied whether exposure of male Wistar rats to BBP during gestation or during the first 21 days of postnatal life, affected testicular size or spermatogenesis in adulthood (90-95 days of age). BBP was administered in the drinking water at a concentration of 1,000 µg/l.

Estimated intake ranged from approximately 125 µg/kg bw/day the two first days after birth to approximately 370 µg/kg bw/day just before weaning based on intended concentrations in drinking water. No analytical confirmation of these estimates was performed. Diethylstilbestrol (DES, 100 µg/l) and an octylphenol polyethoxylate (OPP, 1,000 µg/l) were used as a positive and negative control. The number of treated dams per group ranged from 5 to 6; the males from these litters resulted in approximately 26 to 36 male offspring examined at post-natal day 90-95 for each treatment group. Two studies were performed where the mothers were treated for approximately 8-9 weeks, covering a 2-week period before mating, throughout gestation and up to until 22 days post partum (spanning the whole period of Sertoli cell proliferation). In study 1 exposure to BBP resulted in a small but significant (from 2,014 mg to 1,809 mg) reduction in mean absolute testicular weight and in the mean relative testicular weight (from 4.12 to 3.81 mg/g body weight). In study 2 (which was identical to study 1) the corresponding values were 1,954 to 1,819 mg (absolute testis weight) and 4.09 to 3.82 mg/g bw (relative testis weight). The values using DES was 2,014 to 1,750 mg (study 1) and 1,954 to 1,847 mg (study 2) regarding absolute testis weights, and 4.12 to 3.94 mg/g bw (study 1) and 4.09 to 3.99 mg/g bw (study 2). BBP also caused a reduction (approximately 20%) in daily sperm production (homogenization-resistant spermatids) corresponding to an expected theoretical (not measured) reduction at 2-4% in the number of Sertoli cells. However, in later comments to the study from Sharpe and Turner (1998), they commented that biological variability in these types of studies may have a greater influence on the test results than the test compound tested, however, they still considered the results of the initial study as valid. Due to the negative results in the two later studies (Ashby et al., 1997 and TNO, 1998a,b, see below) the results of the Sharpe et al. (1995) study seems of limited importance for the risk assessment of BBP for developmental effects.

Ashby and coworkers (1997) performed a similar (but not identical) study as Sharpe and coworkers (1995). Ashby et al. (1997) used a greater number of animals in each group, glass bottles and Alpk:Ap$_f$SD rats whereas Sharpe et al., 1995 used smaller number of animals in each group; plastic bottles and Wistar rats. This study was performed to further study the effect on development of the reproductive system after exposure *in utero* and during lactation to low doses of BBP. In this study BBP (purity 98%) was administered in drinking water (1,000 µg/l) to pregnant Alpk:Ap$_f$SD (AP) rats (age between 10 to 12 weeks; 19 rats) during gestation and lactation (the pre-mating exposure period used by Sharpe was omitted). The diet used in this study contained almost half the amount of soya compared to the Sharpe study. The stability of BBP in water feeding bottles was assessed using LC-MS. Controls were given drinking water (19 rats). The sexual development of the pups were monitored until their termination at postnatal day 90 (pnd 90). The BBP dose corresponded to 182.6 µg/kg/day. Pups derived from animals (6 rats) exposed to diethylstilbestrol (DES) in drinking water (50 µg/l, corresponding to 8.6 µg/kg /day) was used as a positive control. The sexual development of the both sexes of pups were monitored. The body weights of the DES pups were significantly reduced at birth, an effect that persisted until pnd 90. The body weight of BBP pups were marginally increased at birth, but no difference was found at pnd 90. DES affected the sexual development of the pups for all endpoints assessed: anogenital distance (AGD) on pnd 2; average day of vaginal opening and prepuce separation; uterus, testes and accessory sex gland weight; cauda epididymis sperm count, and homogenization resistant testicular sperm count at pnd 90. BBP did not affect any of these parameters, with the exception of a 1.1 day advance in the average day of vaginal opening and a small increase in AGD in female pups on pnd 2. These last two effects were considered related to the increased weight of the BBP pups. The incidence of FSH containing cells in the pituitary gland of animals from each dose group was unaffected at pnd 90 (reduced release of FSH during testicular development may cause a reduced testicular growth). The effects observed in

the DES pups are consistent with the results of earlier studies by Sharpe and coworker (1995). However, the absence of an effect of BBP administration on pup testis weight and testicular sperm count on pnd 90 is in contrast to reductions in these parameters reported by Sharpe (1995). The Sharpe (1995) and Ashby (1997) studies differ in the strain of rats used, in the lack of a pre-mating BBP exposure period in the Ashby study, and use of glass feeding bottles in the Ashby and coworkers (1997) study, however, the impact they may have had on the validity and interpretation of the results is probably minor. The report concluded that the small change in the average day of vaginal opening in BBP pups can not alone be taken as evidence of endocrine disruption (Ashby et al., 1997).

A recent TNO study (TNO, 1998a) was performed according to GLP compliance to investigate the effect on the development of male reproductive organs in male F1 pups. The study was conducted under the same protocol as Sharpe and coworkers (1995) with a few enhancements to increase the overall strength of the study. These included larger number of doses, larger sample size, more extensive evaluation of male pups, evaluation of female pups, and analytical characterisation of dosing solutions (stability data of BBP is included in the end of the TNO studies). BBP was given to Wistar rats (28/group) in drinking water at concentrations of 100, 1,000 and 3,000 µg/L.The measured concentrations of BBP analysed in the drinking water were 80, 84 and 86% of the intended level of the low, mid and high dose. BBP was given to F0 females (parents) during the premating period (2 weeks), gestation and lactational period up to weaning on postnatal day (pnd) 21. During these periods the BBP intake ranged from 10-22, 115-229 and 340-674 µg/kg bw/day for the low, mid and high dose group. During the third week of the lactation period the pups started to drink from the water bottles, therefore, the BBP intake in this period may not be correct, since the BBP intake is calculated on the basis of the water consumption and body weights of the dams. Parental females were sacrificed after pnd 23, and F1 pups were sacrificed when males and females were 89-97 and 91-101 days old. A positive control group was given DES 50 µg/L for the same period, however, between gestation day 13 and lactation day 3 the concentration of DES was reduced to 10 µg/L due to animal welfare. No mortality or clinical changes were reported during the study. The body weight, body weight change, food and water consumption of the BBP treated groups was comparable to the control animals for the F0 generation. For the DES group a significant reduction in body weight, body weight change, and food and water consumption was reported during the pre-mating, gestation and lactation period of the F0 females. For the F1 generation body weight, body weight change and food consumption of the males and females of the BBP treatd groups and the females of the DES treated group were comparable to the control group. However, for the F1 males the body weight in the DES treated group was reduced from weaning until sacrifice. No effects on the mating index, female fecundity or fertility were reported. Furthermore, no post-implantation loss was reported. Stillborn pups were reported in 4, 5 and 3 litters of the control, high-dose BBP group and the DES group. The number of stillborn pups was 15, 0, 0, 7 and 13 for the control, low-, mid-, high-dose BBP group and DES group. However, on a litter basis the mean number of live born pups was comparable for all groups, except for the DES group which showed a statistically significant decrease in the number of live born pups per litter. The number of pups found dead or missing (cannibalized by the mother) between pnd 1-4 was 2, 2, 30, 29 and 39 for the control, low-, mid-, high-dose BBP group and the DES group. On a pup basis the increase in pup-mortality from pnd 1-4 in the mid- and high-dose BBP group and DES group was statistically significant compared to control. However, on a litter basis the reduction in the number of live pups was only statistically significant in the DES group. Necropsy of the stillborn and dead pups did not reveal any treatment related effects. The sex-ratio of the pups was comparable in all dose groups. No changes were reported in pup weights compared to controls in the BBP dosed groups, whereas pup weights in the DES group were statistically decreased. Sexual

maturation was not effected in BBP dosed animals, whereas a delay in time of onset of preputial separation was reported in the DES group. Absolute and relative organ weights in the BBP groups were comparable to control animals, whereas absolute testis weight were significantly decreased, and relative weight of liver in males and females and kidney weight in males were increased in the DES group. In the F1 generation analysis of the epididymal sperm production revealed a lower number of sperm in the DES group ($50.9 \cdot 10^6$/3 ml versus $70 \cdot 10^6$/3 ml in control animals), and a slight decrease in the number of normal sperm in the low dose BBP (197.3 versus 198.4 in control animals) and DES group (195.4 versus 198.4 in control animals). However, the authors concluded that the decrease in the number of normal sperm was not considered to be treatment related. No differences in daily sperm production in the testes were reported between the control, BBP and DES groups. The author concluded that the study did not reproduce any of the effects observed by Sharpe and coworkers (1995) at any dose level tested. Specially, no effect was reported on testis weight and daily sperm production.

Due to the stillborn pups and the pups found dead between pnd 1-4 in the control, mid- and highdose BBP groups and the DES group, a follow-up study was performed (TNO, 1998b). In this study BBP was given to Wistar rats at concentrations of 1,000 and 3,000 µg/L. The measured concentrations of BBP analysed in the drinking water were 86 and 77% of the intended level of the mid- and high dose. The female rats were exposed to BBP in the drinking water in the pre-mating period (2 weeks), gestation period and up to sacrifice on pnd 7. The intake dose in the premating period was lower in the follow-up study (109 and 280 µg/kg bw/day for the mid- and high- dose group), compared to the initial study (115 and 340 µg/kg bw/day for the mid- and high-dose group). In the follow-up study the number of stillborn pups was 13, 8 and 28 for the control, mid- and high-dose BBP group. On a pup basis the increased number of stillborn pups was only statistically significant in the high-dose BBP group compared to controls. Furthermore, the number of pups found dead between pnd 1-4 was significantly decreased in the mid-dose BBP group (11 versus 29 in control) and increased in the high-dose BBP group (42 versus 29 in control). However, on a litter basis no effects on pnd 4 were reported. Other effects reported were comparable to the initial study. Necropsy of the stillborn and dead pups did not reveal any treatment related effects. Since the pup mortality in the initial and follow-up study was higher than pup mortality reported in previous dietary study with BBP performed at the same institute, and in studies performed by others (drinking water and diet) this was not considered to be treatment related. Furthermore, pup mortality reported in the control animals in the initial and follow-up study was reported to be higher than the Institute's historical control data. To further evaluate the pupmortality observed in the initial study and follow-up study in control animals and after low dose exposure to BBP in drinking water, a third one-generation study with low exposure to BBP in diet and drinking water was performed.

In this one generation study performed in compliance with GLP and according to OECD Guideline No. 416 Wistar rats (28 per group) were exposed to 1 or 3 ppm BBP (purity 99.2%) in drinking water or in the diet. In females the daily intake of BBP was as following based on daily water or fed intake and body weights; in drinking water; 1 ppm, 0.12 mg/kg bw/day at study start and 0.24 mg/kg bw/day at the end of the study, 3 ppm, 0.35 mg/kg bw/day at study start and 0.80 mg/kg bw/day at end of the study. In diet; at 1 ppm from 0.09 mg/kg bw/day at study start and 0.16 mg/kg bw/day at the end of the study, and at 3 ppm from 0.28 mg/kg bw/day at study start and 0.49 mg/kg bw/day at end of the study. The chemical stability of BBP was assured for a period at least 7 days. The control groups received untreated drinking-water or diet. Females received BBP during the pre-mating (2 weeks), mating (up to 3 weeks) and gestation and lactation period (3 weeks). Males received BBP during the co-housing period only. No clinical or gross pathological findings due to

the treatment and no increase in mortality were reported in the parental animals exposed to BBP. Furthermore, no changes in body weigh or food consumption in the BBP exposed parental animals compared to controls were reported. As regards effects on reproduction parameters, no effects on these parameters were reported in BBP exposed animals (insamination index, fertilisation performance, fertility index, gestation length, number of pups born, birth and pup weight development, litter size, stillbirth, sex ratio, viability and lactation). No enhanced peri-natal pup mortality, or test-substance-related clinical or gross pathological findings were reported in the BBP exposed pups (Bayer AG, 1998).

4.1.2.9.6 Stability of BBP in drinking water

The stability of the BBP in drinking water dosing solutions was determined by UV spectra in a separate study (Monsanto, 1997b). Concentrations of BBP dosing solutions were examined 8 hourrs and 7 days after preparation of a 1 or 3 ppm BBP solution. After 8 hours a 30% or 11% reduction in BBP concentration was reported in the 1 or 3 ppm BBP dosing solution. After 7 days a 40% or 62% reduction was reported in the 1 or 3 ppm BBP dosing solution. In the 1899 initial TNO study the dosing solutions were changed daily, and in the 1975-follow up TNO study every four days. The instability of BBP in the dosing solutions is a problem in all BBP drinking water studies, and has to bee reflected when the results from the studies are evaluated.

4.1.2.9.7 Developmental studies, BBP metabolites, animals

The major metabolites of BBP are mono-*n*-butyl phthalate (MBuP) and mono-*n*-benzyl phthalate (MBeP). Larger quantities of MBuP are formed in rats (44% MBuP versus 16% MBeP) (Eigenberg et al., 1986; Mikuriya et al., 1988). Di-*n*-butyl phthalate (DBP) is metabolised toMBuP (Albro and Moore, 1974; Williams and Blanchfield, 1975; Tanaka et al., 1978). The pattern of malformations produced by MBuP and MBeP were similar (Ema et al., 1995a; Ema et al., 2003) to that produced by BBP (Ema et al., 1992a; 1992b; 1993a) and DBP (Ema et al., 1993b; Ema et al., 1994b). In this section the toxicity of MBuP and MBeP are presented. The section is also supplemented with the more recent developmental toxicity studies performed with DBP. DBP administered during the organogenic and/or late gestation period (Mylchreest et al., 1998) induced an almost similar pattern of malformations in the male reproductive system as were reported after exposure to BBP (Gray et al., 2000; Parks et al., 1999). The supplementations of the DBP studies in the BBP report have not affected the overall conclusion of this section.

The BBP metabolites mono-*n*-butyl phthalate (MBuP, 0, 250, 500 or 625 mg/kg bw/day, Ema et al., 1995a) and mono-*n*-benzyl phthalate (MBeP, 0, 250, 313, 375, 438 or 500 mg/kg bw/day, Ema et al., 1996a) were evaluated for developmental toxicity in Wistar rats (11-15 pregnant rats/group). Rats were exposed once daily to MBuP or MBeP by gastric intubation on day 7-15 of pregnancy and killed on day 20 of pregnancy. After exposure to MBuP a significant decrease in food consumption and a significant reduction in body weight gain in dams was reported from 500 mg/kg bw/day, however, no effects were reported on adjusted body weight gain at 500 and 625 mg/kg bw/day. After exposure to MBeP a significant reduction in food consumption was reported from 250 mg/kg bw/day at pregnancy day 7-15. A significant reduction in weight gain was reported from 313 mg/kg bw/day on pregnancy day 7-15, however, adjusted weight gain was only significantly reduced at 500 mg/kg bw/day. Furthermore, at doses ≥ 313 mg/kg bw/day of MBeP a reddish-brown staining of the facial fur, piloerection and spasticity were reported in the dams.

Embryotoxicity was reported after exposure to doses ≥ 500 mg/kg bw/day of MBuP. These included a significant increase in resorption and dead foetuses per litter, a significant increase in postimplantation loss per litter and live foetuses per litter, and a significant decrease in body weight

of live foetuses. For MBeP embryotoxicity was reported at doses ≥ 438 mg/kg bw/day of MBeP. These included a significant increase in postimplantaion loss per litter and resorption and dead foetuses per litter, and a significant decrease in body weight of live foetuses. Teratogenicity was reported after exposure to doses ≥ 500 mg/kg bw/day of MBuP. These included a significant increase in foetuses per litter with external malformations (cleft palate), skeletal malformations, and internal malformations (dilatation of renal pelvis). For MBeP teratogenicity was reported at doses ≥ 313 mg/kg bw/day of MBeP. These included a significant increase in foetuses per litter with skeletal malformations, and internal malformations (dilatation of renal pelvis from 375 mg/k bw/day). Maternal and developmental NOAEL was 250 mg/kg bw/day for MBuP, and the maternal LOAEL and developmental NOAEL was 250 mg/kg bw/day for MBeP. The pattern of malformations produced by MBuP and MBeP were similar to that produced by BBP, suggesting that MBuP or MBeP and/or its possible metabolic products may be responsible, at least in part, for the teratogenic effect of BBP.

The developmental toxicity of MBeP was studied in Wistar rats (12 weeks of age) (Ema et al., 1996b). Pregnant rats (10 to 17 rats per group) were given MBeP by gastric intubation at 375, 500 or 625 mg/kg on days 7-9, or 250, 375, 500 or 625 mg/kg on days 10-12 or 13-15 of pregnancy. The pregnant rats were killed on day 20 of pregnancy. *Results after administration on pregnancy day 7-9*; Three of 15 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain from day 7-10 in the 375, 500 and 625 mg/kg dose groups and on day 10-20 in the 625 mg/kg dose group was significantly reduced, compared to the control group. A decreased food consumption was reported in the same dose groups as well. The adjusted weight gain was only reduced in the 375 and 625 mg/kg dose groups, compared to control animals. Complete resorption of all implanted embryos was reported in 9 of the 12 litters in the 625 mg/kg dose group. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. A significantly reduction in female offspring body weight was reported from 375 mg/kg, and in male offspring from 500 mg/kg. No external malformations were reported. Skeletal examination revealed a significantly increased incidence of skeletal malformations in the 625 mg/kg dose group on a per litter basis compared to control animals. These malformations included fusion of cervical or thoracic vertebral arches, fusion of thoracic vertebral bodies, absence of lumbar vertebral arches and fusion or absence of ribs. No increased incidence of internal malformations was reported. *Results after administration on pregnancy day 10-12*; Two of the 12 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain on days 10-13 and days 13-20 in the 500 and 625 mg/kg dose groups was significantly reduced, compared to the control group. The adjusted weight gain was significantly lower in the 250, 500 and 625 mg/kg dose groups compared to control animals. The food consumption on days 10-13 in the 250, 375, 500 and 625 mg/kg dose groups and on days 13-20 in the 250, 500 and 625 mg/kg dose groups was significantly reduced compared to the control group. Complete resorption of all implanted embryos was reported in 3 of the 11 litters in the 500 mg/kg dose group and in 7 of 10 litters in the 625 mg/kg dose group. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. A significantly reduction in female offspring body weight was reported from 500 mg/kg, and in male offspring at 625 mg/kg. There was no significant difference in the incidence of external, skeletal, and internal malformations between the groups exposed to MBeP or the control group. *Results after administration on pregnancy day 13-15;* Four of 17 pregnant rats died in the 625 mg/kg dose group. The maternal body weight gain on day 13-16 and days 16-20 in the 500 and 625 mg/kg dose groups was significantly reduced, compared to the control group. The adjusted weight gain was significantly lower in the 250, 375, 500 and 625 mg/kg dose groups compared to control animals. The food consumption on days 13–16 in all dose groups and on days 16—20 in the 625 mg/kg dose group

was significantly reduced compared to control animals. Complete resorption of all implanted embryos was reported in 5 of the 14 litters at 500 mg/kg, and 11 of 13 litters at 625 mg/kg. The incidence of postimplantation loss per litter in the 500 and 625 mg/kg dose group was significantly higher than in the control group. No significant reduction in male or female offspring body weight was reported. The incidence of foetuses with external malformations in the 500 mg/kg dose group was significantly higher compared to control animals, and all of these foetuses had cleft palate. A significantly increased incidence of foetuses with skeletal malformations was reported from 375 mg/kg, and all of these foetues had fusion of the sternebrae. No significantly increased incidence of foetuses with internal malformations was reported. These results indicate that the susceptibility and spectrum of the developmental toxicity of MBeP vary with the developmental stage at the time of administeration, as was also reported after administration of BBP during different days of pregnancy.

Ema et al. (2003) studied the effect of monobenzyl phthalate MBeP, a major metabolite of BBP on the development of the reproductive system in rats. He also looked into the role of MBeP in the antiandrogenic effects of BBP. In this study pregnant Wistar rats (16/dose group) were given MBeP by gavage at doses of 167, 250 and 375 mg/kg bw/day on gestation day (gd) 15 to 17 Foetuses were examined on gd 21. Maternal body weight gain on gd 15-18 was significantly decreased from 167 mg/kg bw/day (31, 24, 23 and 15g in the control, 167, 250 and 375 mg/kg bw/day dose group). Maternal food consumption was significantly decreased on gd 15-18 from 167 mg/kg bw/day (54, 46, 40 and 33 in the control, 167, 250 and 375 mg/kg bw/day dose group). The adjusted maternal weight gain was significantly decreased from 250 mg/kg bw/day. Foetal weight was significantly decreased at 375 mg/kg bw/day. A significant increase in the incidence of undescended testes/litter was reported from 250 mg/kg bw/day [2(2), 1(1), 21(12) and 79(16) in the control, 167, 250 and 375 mg/kg bw/day dose group]. A decrease in anogenital distance (AGD) and ratio of AGD on the cube root of body weight was reported in male foetuses at 250 mg/kg bw/day. No effect on AGD was found in female foetuses. The study indicated that MBeP produced adverse effects on the development of the reproductive system in male offspring and suggested that MBeP may be responsible for the antiandrogenic effects of BBP.

The effect of prenatally exposure to Monobutyl phthalate (MBuP) on testicular descent was studied in Wistar-King A rats (Imajima et al., 1997). Pregnant rats (7 per group) were exposed to MBuP (300 mg/day equivalent to approximately 1,000 mg/kg bw/day) or solvent (sesame oil) by gavage from gestation day (gd) 15 to 18. Male offspring was evaluated on gd 20 and on postnatal day (pnd) 30-40 to determine the position of the testes. On gd 20 all the testes were located in the lower abdominal cavity near the bladder neck in the controls (n = 19 from three litters), however, in offspring treated *in utero* to MBuP the testes were located significantly higher in the abdominal cavity, and some were located near the kidney (n = 15 from three litters). On pnd 30-40, in the control group, all testes descended into the scrotum and the incidence of cryptorchidism was 0% (n = 15 from three litters), whereas, in the MBuP treated offspring (n = 26 from five litters), 22 rats showed cryptorchidism (14 unilateral and 8 bilateral undescended testes), and the incidence of cryptorchidism was 84.6%. Twenty six of the total 30 undescended testes (86.7%) were located in the abdominal cavity and the remaining four (13%) were located at the external inguinal ring. This may indicate that MBuP act in an anti-androgenic manner, since testis descent is under androgenic control.

The time-specific effects of monobutyl phthalate (MBuP) on the transabdominal migration of the testis were studied in fetal rats (Shono et al., 2000). In this study three groups of pregnant Wistar-King A rats were administered MBuP by stomach feed tubing (0.3 g/day, corresponding to approximately 1,000 mg/kg bw/day), group 1 (2 rats) from gestation day (gd) 7-10, group 2 (2 rats) from gd 11-14, and group 3 (6 rats) from gd 15-18. The control group (group 4, 5 rats) was given

vehicle (sesame oil) only from gd 7-18. At gd 20 the fetuses were obtained by Caesarean section, and the position of the testes were determined in all groups. Furthermore, in group 3 and 4 the testis, epididymis, cranial suspensory ligament and gubernaculum were examined under a dissecting microscope, and the levels of testosterone were measured. The degree of the transabdominal testicular migration was determined by measuring the distance from the bladder neck to the lower pole of the testis. The results from this study showed that in the control group all 30 testes were anchored at the bottom of the abdominal cavity near the bladder neck by a swollen gubernaculum, whereas in group 3 (exposure to MBuP from gd 15-18) the testes were high in the abdominal cavity and associated with both an elongated gubernaculum and a hypertrophic cranial suspensory ligament. The mean transabdominal testicular migration values in group 1, 2, 3 and 4 were 12.3 ± 5.9 (10 testes), 24.5 ± 5.2 (10 testes), 57.9 ± 2.6 (38 testes) and 9.3 ± 1.9 (30 testes). Values were significantly higher in group 2 and 3 compared to the control group. Histopathologic examination showed a poorly developed epididymis in group 3, with a small thin ductus deferens, although there were no remarkable changes in the morphological features of Sertoli and Leydig cells. The mean testosterone levels were 50.9 ± 3.8 pg/testis in MBuP treated fetuses (25 testes) and 852± 80.3 pg/testis in the control fetuses (30 testis), the levels was significantly lower in the MBuP treated rats compared to the controls.

A comparative developmental study was performed with BBP and Di-*n*-butyl phthalate (DBP) in Wistar rats. Pregnant rats were given either BBP or DBP by gastric intubation at a dose of 750, 1,000 and 1,250 mg/kg bw/day on days 7-9, days 10-12, and days 13-15 of pregnancy (12-13 pregnant rats/group). No information was provided regarding the evaluation of maternal effects. Regardless of the days of treatment, a significantly increased incidence of post-implantation loss per litter, and decreased number of live foetuses per litter was found at all doses of BBP and DBP. A significant decrease in foetal weight was reported in all dose groups after exposure on gd 7-9. After exposure on gd 10-12 a significant decrease in foetal weight was reported at 1,000 mg/kg bw/day of BBP and 1,250 mg/kg bw/day of DBP, whereas no decrease in foetal weight was reported after exposure on gd 13-15. While treatment with BBP and DBP at doses of 750 mg/kg bw/day and above on days 7-9 or days 13-15 resulted in a significant increase in the incidence of malformations, no increase in the incidence of malformed foetuses was found after treatment with BBP or DBP on days 10-12. These malformations included external malformations (cleft palate) after exposure on gd 13-15, and skeletal malformations (fusion of sternebrae, fusion and absence of cervical vertebral arches, thoracic vertebral arches and bodies, or fusion and absence of ribs) after exposure on gd 7-9 or 13-15. The author concluded from this study that the similarity in the dependence of the gestations days of treatment on the manifestation of developmental toxicity, and on the spectrum of foetal malformations caused by BBP and DBP, they may act by the same mechanism, possibly via a common metabolite of these two parent compounds. The developmental NOAEL was 750 mg/kg bw/day of BBP in this study (Ema et al., 1995b).

The effects of DBP on prenatal and early neonatal development of the reproductive tract in rats were studied *in vivo* (Mylchreest et al., 1998). In this study a markedly disturbed development of the male reproductive tract (internal and external) in rat offspring exposed via their mothers during gestation and lactation, was observed at all dose levels (250, 500 or 750 mg/kg bw/day) in the absence of significant maternal toxicity. In female offspring sporadic cases of reproductive tract malformations were observed at 500 and 750 mg/kg bw/day. Age at vaginal opening and estrus cyclicity were not affected. The results of this study suggested that DBP does not possess estrogenic activity but rather shows anti-androgenic-like activity at these dose levels. The results reported in the Mylchreest et al. (1998) study were confirmed in a study by Ema et al. (1998b). In this study Wistar rats were exposed to DBP, 331, 555 or 661 mg/kg bw/day at day 11-21 of pregnancy.

Comparative embryotoxicities of butyl benzyl phthalate, mono-*n*-butyl phthalate and mono-*n*-benzyl phthalate in mice and rats: *in vivo* and *in vitro* was studied by Saillenfait et al. (2003). For study description and results see Section 4.1.2.9.

4.1.2.9.8 Developmental studies, BBP, humans

In a study by Swan et al. (2005) they examined the anogenital distance (AGD) and other genital measurements (smaller genitalia and undescended testis) in 85 boys 2-30 month of age in relation to prenatal phthalate exposure in humans. The anogenital index (AGI) was defined (AGD divided by weight examinations) and the age adjusted AGI was calculated by regression analysis. *Results:* The urinary concentration of four phthalate metabolites MEP (mono-ethyl-phthalate, reflecting exposure to DEP), MBuP (mono-n-butyl-phthalate, reflecting exposure to DBP), MBeP (mono-benzyl-phthalate, reflecting exposure to BBP) and MiBP (mono-isobutyl-phthalate, reflecting exposure to DiBP) were inversely related to AGI. When comparing boys with prenatal MBeP concentrations the odds ratio for a shorter than expected AGI was 3.8. For the other phthalate metabolites the odds ratios were as following: 10.2 for MBuP, 4.7 for MEP, and 9.1 for MiBP (all *p*-values < 0.05). The degree of testicular descent was associated with AGD. The proportions of boys with one or both testicles incompletely descended were 20%, 9.5% and 5.9% for boys classified as having short, intermediate and long AGI. A short AGI (25 boys) was defined as an AGI below the 25th percentile for age. This group had an AGI that was on agerage 18.3% (range 10-32%) shorter than expected. Boys (n = 17) with AGI ≥ 75th percentile of expected were classified as having a long AGI, and boys (n = 43) with AGI between the 25th and 75th percentile of expected were considered intermediate. The boys age and weight did not differ appreciably among these groups. AGD was also significantly associated with penile volume; R = 0.27 (p = 0.001) and penile volume divided by weight was correlated with AGI (R = 0.43, p = 0.001). The summary phthalate score was also defines for the quantification of joint exposure to these four phthalate metabolites. The age adjusted AGI decreased significantly with increasing phthalate score (*p*-value for slope = 0.009). The median concentrations of phthalate metabolites that were associated with a shorter AGI and incomplete testis descent were found to be below those phthalate concentrations measured in urine in one-quarter of the female populations in USA (CDC, 2003). These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, only 85 boys, further studies with larger sample size have to be performed before clear conclusions can be drawn from this study.

In a study by Main et al. (2005) they investigated whether phthalate monoester metabolite contamination of human breast milk had any influence on the postnatal surge of reproductive hormones in newborn boys as a sign of testicular dysgenesis. Biological samples were obtained from a prospective Danish-Finnish cohort study on cryptorchidism 1997-2001. The individual breast milk samples were collected 1-3 month postnatally (n = 130, 62 cryptorchid/68 healthy boys), and were analysed for phthalate monoester metabolites; MBeP (mono-benzyl phthalate, reflecting exposure to BBP), MME (mono-methyl phthalate, reflecting exposure to DMP), MEP (mono-ethyl-phthalate, reflecting exposure to DEP), MBuP (mono-n-butyl phthalate, reflecting exposure to DBP), MEHP (mono-2-ethylhexyl-phthalate, reflecting exposure to DEHP), and MINP (mono-isononyl phthalate, reflecting exposure to DINP). Serum samples (obtained in 74% of all boys) were analysed for gonadotropins, sex-hormone binding globuline (SHBG), testosterone, and inhibin B. *Results:* All phthalate monoester metabolites were found in breast milk samples with large variations. The medians (minimum – maximum) levels in µg/L were; MBeP 1.2 (0.2-2.6), MME 0.10 (< 0.01-5.53), MEP 0.95 (0.07-41.4), MBuP 9.6 (0.6-10,900), MEHP 11 (1.5-1,410), and MINP 95 (27-469). No association was found between phthalate monoester levels in breast milk and cryptorchidism. However, MEP and MBuP showed positive correlations with SHBG

(r=0.323, p 0.002 and r=0.272, p=0.01, the value for MBeP was r=0.188, p=0.074), MMP, MEP and MBuP with LH/free testosterone ration (r=0.21 to 0.323, p 0.002 to 0.044, the value for MBeP was r=0.06, p=0.57), and MINP with LH (r=0.243, p 0.019, the value for MBeP was r=0.049, p=0.643). MBuP was negatively correlated with free testosterone (r=0.22, p=0.033, the value for MBeP was r=-0.07, p=0.951). The other phthalate monoesters including MBeP showed similar (as indicated above), however, not significant tendencies.

4.1.2.9.9 Summary developmental studies, BBP and BBP metabolites, animals

In the developmental toxicity studies in rats and mice after exposure to BBP or its major metabolites (MBuP or MBeP) developmental toxicity in offspring included prenatal mortality, reduced fetal weight, and malformed foetuses. Maternal toxicity was characterised as reduced body weight gain and increased liver weight, accompanied by a decreased food consumption. The determined NOEL/NOAEL/LOAEL values for maternal toxicity and developmental toxicity derived from the various studies are given in **Table 4.28**.

Study Design	Effect Level	Critical Effect	Reference
BBP			
Swiss DC-1 mice and Sprague-Dawley rats; 27-30/group; Administration in diet on gd 6-15; 0.1, 0.5, and 1.25% mice (182, 910, 2,330 mg/kg/day mice) and 0.5, 1.25 and 2.0% rats (419, 1,102 and 1,641 mg/kg/day rats)	NOAEL mice maternal 182 mg/kg bw/day and NOAEL offspring 182 mg/kg/day NOAEL rat maternal and offspring 419 mg/kg/day	Mice: At 910 mg/kg/day a slight reduction in dam weight gain (15%), no reduction in adjusted body weight gain, prenatal mortality and malformed fetuses at doses ≥ 910 mg/kg/day. Rat: At 1,102 mg/kg/day reduced dam weight gain. At 1,641 mg/kg/day reduced dam and foetal weight gain, and increased resorption and malformations.	NTP (1990) (mice). NTP (1989) (rats).
Cpb-WU pregnant rats; Administration of BBP by gavage gd 6-15 or 6-20; 0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg/day; 25/group in the 0, 450, 750 and 1,250 mg/kg/day dose group, 10/group in the 270, 350, 580, 970, 1,600 and 2,100 g/kg/day dose group	NOAEL maternal 450 mg/kg/day (exp. gd 6-20) and 580 mg/kg/day (exp gd 6-15) NOAEL offspring 270 mg/kg/day (exp gd 6-20) and 350 mg/kg/day (exp.gd 6-15)	Maternal; increased liver weight at 580 mg/kg/day (exp. gd 6-20) and at 750 mg/kg/day (exp. gd 6-15). Offspring; decreased relative testis weight at 270 mg/kg/day (exp. gd 6-20). Effects on testicular migration at 580 mg/kg/day (more pronounced after long exp.), reduced fetal weight from 350 mg/kg/day (exp. gd 6-20) and from 450 mg/kg/day (exp. gd 6-15).	Piersma et al. (2000)
CD Sprague-Dawley rats; 2-generation study; 30/sex/group; administration in feed; 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day.	NOAEL for developmental effects: 50 mg/kg bw/day based on reduced anogenital distance from 250 mg/kg bw/day in F1 and F2 offspring. NOAEL for maternal toxicity: 250 mg/kg bw/day	Development: reduced anogenital distance (absolute and adjusted) from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day. Maternal toxicity: organ weight changes, and histopahological lesions in the liver graded as minimal in females at 750 mg/kg bw/day.	Tyl et al. (2004)
Pregnant Sprague-Dawley rats; (no information about number); administration by gavage; 0 or 750 mg/kg bw/day from gd 14 through pnd 3.		Maternal toxicity: no information. Offspring: on pnd 2 AGD and testis weight was decreased, and on pnd 13 the incidences of areolas were increased for pups exposed *in utero* to BBP.	Parks et al. (1999)

Study Design	Effect Level	Critical Effect	Reference
BBP			
Sprague-Dawley rats; two-generation study; 25/sex/group; administration by gavage; 0, 20, 100 and 500 mg/kg bw/day	NOAEL: 20 mg/kg bw/day for developmental effects based on decreased body weight in F1 offspring from 100 mg/kg bw/day.	Fo; decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovaryweight in females at 500 mg/kg/day. A decrease in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD (absolute) was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day. BBP did not affect reproductive ability, including delivery and lactation. F2: no significant effects related to BBP exposure up to pnd 21.	Nagao et al. (2000)
Pregnant 0F1 mice 15-23/group, single oral dose on gd 8 of BBP: 0, 280, 560, 1,120, 1,690 mg/kg. MBuP: 0; 200, 400, 800, 1,200 mg/kg. MBeP: 0, 230, 460, 920, 1,380 mg/kg. PregnantSprague-Dawley rats 7-13/group, single oral dose on gd 10 of BBP: 0, 280, 560, 1,120, 1,690 mg/kg. MBuP: 0;200, 400, 800, 1,200 mg/kg. MBeP: 0, 230, 460, 920, 1,380 mg/kg		Mice; BBP, MBuP and MBeP: Decreased body weight gain at the highest dose levels, however, no changes in corrected body weight gain. A reduction in live foetuses/litter was reported in the higehst dose groups. An increase in resorptions/litter in the highest dose groups. A dose-depended increase in malformed foetuses/litter was reported from 560 mg/kg BBP, 200 mg/kg MBuP and 920 mg/kg MBeP. Rat; BBP, MBuP and MBeP: No decrease in maternal body weight gain. No effects on live foetuses/litter and on post-implantation loss/litter were reported. A slight increase in malformed foetuses/litter was reported from 1,120 mg/kg BBP.	Saillenfait et al. (2003)

Study Design	Effect Level	Critical Effect	Reference
BBP			
Pregnant Sprague-Dawley rats 5/group; administration by gavage; corn oil or 750 mg/kg/day from gd 14 through postnatal day 3.		Maternal toxicity: no information. Offspring; 84% showed malformations in the testis, epididymis, accessory reproductive organs and external genitalia at 3-4 month of age. Reduced anogenital distance, decreased testis, seminal vesicle, ventral prostate and epididymis weight at day 2 of age, and males with areolas at day 13 of age.	Gray et al. (2000)
Wistar rats; Administration in diet on day 0-20 of pregnancy; 0.25, 0.5, 1.0 and 2.0% (185, 375, 654 and 974 mg/kg bw/day).	NOAEL maternal: 375 mg/kg bw/day NOAEL offspring: 185 mg/kg bw/day	At doses ≥ 375 mg/kg bw/day reduced body weight gain and at doses ≥ 654 mg/kg/day reduced adjusted weigth gain in dams. At 375 mg/kg/day reduced number of foetuses per litter. At 645 mg/kg /day reduced body weight in foetuses. At 974 mg/kg bw/day complete resorption.	Ema et al. (1990)
Wistar rats; Administration in diet on day 0-20 of pregnancy; 2% (974 mg/kg/day); Pair-feed pregnant rats.		At 974 mg/kg/day complete resorption and reduced body weight and adjusted body weight gain in dams. Pair fed rats showed the same reduction in body weight gain. No other effects in pair-feed pregnant rats.	Ema et al. (1991)
Wistar rats; Gastric intubation on day 7-15 of pregnancy; 500, 750 and 1,000 mg/kg/day.	LOAEL maternal: 500 mg/kg bw/day NOAEL offspring: 500 mg/kg bw/day	At 500 mg/kg/day reduced food consumption in dams. At 750 mg/kg/day reduced food consumption and body weight gain in dams, complete resorption in some dams, decreased foetal weight, malformations. At 1,000 mg/kg/day reduced adjusted body weight gain, high maternal mortality, complete resorption in all dams.	Ema et al. (1992a)
Wistar rats; Administration in diet on day 0-20, 0-7, 7-16 and 16-20 of pregnancy; 2% in the diet (974 mg/kg bw/day); Pair-feed pregnant rats.		Postimplantation loss was increased after exposure on day 0-20, 0-7, 7-16. Teratogenicity was reported after exposure on day-16-20. No effects in pair-feed pregnant rats.	Ema et al. (1992b)
Wistar rats; Administration through diet on day 0-20, 0-11 and 11-20 of pregnancy; 2% in the diet (974 mg/kg bw/day); Pair-feed pregnant rats.		Reduced body weight gain and adjusted body weight gain in dams in all groups. Complete resorption after exposure on day 0-20, 0-11. Teratogenic effects after exposure on day 11-20. No effects in pair-feed pregnant rats.	Ema et al. (1992c)

Study Design	Effect Level	Critical Effect	Reference
BBP			
Wistar rats; Administration once daily by gastric intubation on days 7-9, 10-12 and 13-15 of pregnancy; 600, 750 and 1,000 mg/kg bw/day.	NOAEL offspring: 600 mg/kg bw/day	At doses ≥ 750 mg/kg bw/day on day 7-9 or at 1,000 mg/kg bw/day on day 10-12 foetus weight decrease. At 1,000 mg/kg on exposure day 10-12 litters totally resorbed was increased. At doses ≥ 750 mg/kg bw/day on exposure day 7-9 and 13-15 increased malformations.	Ema et al. (1993a)
Wistar rats; Administration in diet on day 0 through sacrifice on day 7, 9 or 11 of pregnancy; 2% (974 mg/kg bw/day); Pair fed pregnant rats.		Increased postimplantation loss in rats killed on day 11. Regardless of day of sacrifice the ovarian and uterine weights and plasma progesterone levels were decreased in BBP treated rats. No effects in pair-feed pregnant rats.	Ema et al. (1994a)
Wistar rats; 10-14 pregnant rats/groups, 11-13 pseudopregnant rats/group; Gastric intubation on day 0-8 of pregnancy; 250, 500, 750 and 1,000 mg/kg bw/day.	LOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 250 mg/kg bw/day decreased maternal body weight gain. At doses ≥ 500 mg/kg bw/day decreased foetal body weight. At ≥ 750 mg/kg bw/day decrease in implantations per rat. At doses ≥ 500 mg/kg bw/day decreased uterine growth and serum progesterone levels in pseudopregnant rats. No effects on adjusted body weight gain.	Ema et al. (1998a)
Pregnant Wistar rats 16/group; administration by gavage; olive oil, 250, 500 or 1,000 mg/kg from gd 15 to 17.	NOAEL maternal: 250 mg/kg NOAEL offspring: 250 mg/kg	Maternal toxicity: Reduced body weight gain and food consumption from 500 mg/kg. No effect on adjusted body weight gain. Decrease in the number of live foetus/litter, and decreased foetal body weight at 1,000mg/kg. Decrease in AGD in male offspring, and increase in the incidence of undescended testis from 500 mg/kg.	Ema et al. (2002)
Wistar rats; 9-16 pregnant rats/group; Administration by gavage on gd 11,12 and 13, killed on gd 20; 0, 250, 1,000, 1,500 and 2,000 mg/kg/day.		At doses ≥ 1,000 mg/kg/day maternal toxicity. Decreased foetal weight at doses ≥ 1,500 mg/kg/day, gross anomalies at 1,500 and 2,000 mg/kg/day; skeletal anomalies at 1,000 mg/kg/day. A tendency to increased maternal metallothionein at 2,000 mg/kg/day. No measurements of plasma and tissue zinc concentrations.	Keen (1998), Draft

Study Design	Effect Level	Critical Effect	Reference
BBP			
Wistar rats; 26-34 female/group; exposure 2 weeks before mating, throught gestation, and 22 days post partum; Administration in drinking water; 1mg/L (0.125 mg/kg bw/day first day - 0.370 mg/kg bw/day before weaning).		Small reduction in absolute and relative testes weight, reduced daily sperm production	Sharpe et al. (1995)
Alpk:ApfSD (AP-rats) 19 female/group; exposure through gestation and up to post natal day 90; Administration in drinking water; 1 mg/L (0.186 mg/kg bw/day).		No critical effects in pups on testicular weights and testicular sperm counts.	Ashby et al. (1997)
Wistar rats (28 female/group); exposure through gestation and up to post natal day 21; Administration in drinking water, 100, 1,000 and 3,000 µg/L (0.01-0.022, 0.115-0.229 and 0.340-0.674 mg/kg bw/day).		No effects on pups regarding the reproductive system. No maternal toxicity. High incidence in pup-mortality in control and BBP exposed pups.	TNO (1998a)
Wistar rats (28 females/group) exposure through gestation and up to post natal day 7; Administration in drinking water, 1,000 and 3,000 µg/L (0.109 and 0.28 mg/kg bw/day).		No effects on pups regarding the reproductive system. No maternal toxicity. High incidence of pup-mortality in control and BBP exposed pups.	TNO (1998b)
Wistar rats (28 females/group), exposure through premating (2 weeks), mating (3 weeks), gestation and lactation (3 weeks). Administration in drinking water, 1 and 3 ppm (0.12-0.24 and 0.35 to 0.8 mg/kg/bw/day); in diet 1 and 3 ppm (0.09-0.16 and 0.28-0.49 mg/kg bw/day).		No effects on pups regarding the reproductive system. No maternal toxicity. No increase in pup-mortality in control animals compared to historical data or in BBP exposed pups.	Bayer (1998)

Study Design	Effect Level	Critical Effect	Reference
BBP metabolites, MBuP and MbeP			
Wistar rats; MBuP; Gastric intubation on pregnancy day 7-15; 250, 500 and 625 mg/kg bw/day.	NOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 500 mg/kg bw/day reduced food consumption and weight gain in dams, increased resorption, dead foetus and postimplantation loss per litter. Increased malformations.	Ema et al. (1995a)
Wistar-King A rats; MBuP; Administration by gavage on gd 7-10 (2 pregnant rats), on gd 11-14 (2 pregnant rats) on gd 15-18 (6 pregnant rats); Approx. 1,000 mg/kg bw/day, control rats (5 pregnant rats) received sesame oil from gd 7-18.		Maternal toxicity: No information. Offspring: on gd 20 the testis was located significantly higher in the abdominal cavity after exposure on gd 11-14 and 15-18, compared to controls. The testosterone levels were significantly lower in MBuP treated fetuses compared to control fetuses.	Shono et al. (2000)
Wistar-King A rats; MBuP; 7/group; Administration by gavage on gd 15-18; 0 and approx. 1,000 mg/kg bw/day		Maternal toxicity: no information. Offspring: on gd 20 testis were located higher in the abdominal cavity compared to control pups. On pnd 30-40 the incidence of cryptorchidism was 84.6% in exposed animals and 0% in the control group.	Imajima et al. (1997)
Wistar rats; MBeP; Gastric intubation on pregnancy day 7-15; 250, 313, 375, 438 and 500 mg/kg bw/day.	LOAEL maternal: 250 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	At doses ≥ 250 mg/kg bw/day reduced food consumption. From 313 mg/kg bw/day reduced weight gain, at 500 mg/kg bw/day reduced adjusted weight gain and maternal toxicity. At doses ≥ 313 mg/kg bw/day malformations. At doses ≥ 438 mg/kg bw/day embryotoxic effects.	Ema et al. (1996a)
Wistar rats; MBeP; Gastric intubation on pregnancy day 7-9, 10-12 or 13-15; 250, 375, 500 and 625 mg/kg bw/day		Significantly increased incidence of postimplantation loss at 500 mg/kg regardless of days of treatment. From 375 mg/kg a significantly increased incidence of teratogenic effects at exposure on pregnancy day 7-9 or13-15. No teratogenic effects on exposure day 10-12.	Ema et al. (1996b)
Wistar rats; MBeP; Gastric intubation on pregnancy day 15-17.; 167, 250 and 375 mg/kg bw/day.	LOAEL maternal: 167 mg/kg bw/day NOAEL offspring: 250 mg/kg bw/day	Significantly decreased foetal weight gain at 375 mg/kg bw/day. Significant increase in the incidence of undescended testis from 250 mg/kg bw/day. Decreased AGD from 250 mg/kg bw/day. Significantly decreased maternal food consumption and weight gain from 167 mg/kg bw/day. Significantly decreased adjusted maternal weight gain from 250 mg/kg bw/day.	Ema et al. (2003)

The developmental toxicity of BBP and its major metabolites MBuP and MBeP have evaluated whether or not embryotoxicity (lethality) or teratogenicity was observed in the presence or absence of maternal toxicity. In several developmental toxicity studies in rats (NTP, 1989; Ema and coworkers, 1990 and the following Ema et al., studies; Gray et al., 2000; Parks et al., 1999; Piersma et al., 2000; Nagao et al., 2000; Tyl et al., 2004) indications of maternal effects, when reported, such as reduced weight gain, increased liver or kidney weight, and reduced food consumption, were observed at doses higher, equal or below BBP doses that produced developmental toxicity. In a new 2-generation study (Tyl et al., 2004) the NOAEL for effects in offspring was 50 mg/kg bw/day based on a dose-related significant reduction in anogenital distance (AGD) in both the F1 and F2 male offspring from 250 mg/kg bw/day. When the AGD values were adjusted for individual body weights (by analysis of covariance, ANCOVA with body weight as the covariate) the AGD was still significantly reduced compared to the control group. A reduction in AGD at birth is one of the most sensitive indicators of anti-androgenic activity. At 750 mg/kg bw/day a significant increase in F1

and F2 male pups with one or more nipples and/or areolae were reported. Furthermore, at 750 mg/kg bw/day in the F1 generation a reduction in reprodudctive organ weights (testis, epididymis, prostate, and seminal vesicle), and a significant increase in the number of rats with histopathological changes in the reproductive organs were reported. In the F2 male offsprings at weanling necropsy reduced testis weight, and a significant increase in gross lesions in the reproductive organs was reported at 750 mg/kg bw/day. The NOAEL for maternal toxicity was 250 mg/kg bw/day based on organ weight changes (liver and kidney) and histopathological lesions graded as minimal in the liver at 750 mg/kg bw/day. In this 2-generation study BBP was administered in the feed to CD (Sprague-Dawley) rats 30/sex/group at doses of 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day. In the four recently published studies (Piersma et al., 2000; Gray et al., 2000; Parks et al., 1999; Ema et al., 2002) pregnant rats were exposed to BBP by gavage during the organogenic period and/or the late prenatal early postnatal period. In the study performed by Piersma and coworkers (2000) pregnant rats were exposed to BBP *in utero* from gestation day 6 to 20 (long exposure) or 6 to 15 (short exposure), and necropsied on day 21. BBP induced reduced testicular weight in the offspring from 270 mg/kg bw/day, and reduced fetal weight from 350 mg/kg bw/day with a NOAEL at 270 mg/kg bw/day (exposure gd 6-20). Retarded transdominal descent of testis was reported with a NOAEL of 450 mg/kg bw/day. This effect was more pronounced after exposure on gd 6-20. The maternal NOAEL (exposure gd 6-20) was 450 mg/kg bw/day and the NOAEL (exposure gd 6-15) was 580 mg/kg bw/day based on increased liver weight. In the study performed by Gray and coworkers (2000) pregnant rats were given 750 mg/kg bw/day of BBP from gestation day 14 through postnatal day 3. This dose regime induced malformations in the testis, accessory reproductive organs and genitalia in 84% of male offspring at 3-4 month of age. Furthermore, reduced AGD on post-natal day 2 and areolas on post-natal day 13 were additionally seen in male offspring. The study by Parks et al. (1999) used the same dose regime as Gray and coworkers. In this study reduced AGD and testis weight was reported on post-natal day 2, and areolas on post-natal day 13. In the study by Ema et al. (2002) a decrease in AGD and an increase in the incidence of undescended testis was reported in male offspring exposed to 500 or 1,000 mg/kg BBP from gd 15-17. In the recent two-generation study (Nagao et al., 2000) BBP was administered by gavage (0, 20, 100 and 500 mg/kg bw/day). In this study a significant reduction in fetal body weight was reported at 100 and 500 mg/kg bw/day on pnd 0. Furthermore, in male offspring (preweanling rats) a reduction in AGD (absolute), tesist weight, epididymis weight, decreased FSH level and number of spermatogonia and spermatocytes in the seminiferous tubules was reported at 500 mg/kg bw/day. Since birth weight was reduced at 100 mg/kg bw/day in this study, it is unclear whether analysis of adjusted AGD would have shown decreased AGD at 100 mg/kg bw/day. In postweanling rats at 500 mg/kg bw/day a decreased body, testis and epididymis weight were reported. Furthermore, at 500 mg/kg bw/day, a delay in preputial separation in males, decreased testosterone and LH levels and increased incidence of testicular athrophy with decreased number of germ cells in the seminiferous tubules and decreased number of sperm in the epididymis were reported. The only maternal effects reported in this study was a significant increase in kidney weight (relative and absolute) at 100 and 500 mg/kg bw/day, and a decrease in ovaries weight (absolute and relative) at 500 mg/kg bw/day. In pregnant rats exposed to BBP from gestation day 6 to 15, maternal toxicity evident as reduced weight gain, and/or increased liver weight, and reduced food consumption was reported in the presence of developmental toxicity evident as reduced foetal weight and malformed foetuses (NTP, 1989 (administration in diet); Ema et a., 1992a (administration by gavage). However, in the recent studies performed by Tyl et al. (2004) (administration in diet), and Piersma and co-workers (2000) (administration by gavage) developmental toxicity evident as reduced absolute and adjusted AGD in F1 and F2 offspring (Tyl et al., 2004), and reduced fetal and testicular weight in offspring (Piersma et al., 2000) were reported in the absence of maternal toxicity.

In mice BBP induced malformed foetuses at a dose level (910 mg/kg bw/day) which only induced maternal effects in the form of a slightly reduced (15%) absolute body weight gain (NTP, 1990). No effects on the body weight gain were observed when the weight of the dams was adjusted for the weight of the gravid uterus.

To further study the embryolethality/teratogenic effects of BBP in rats, Ema and co-workers (1992b; 1992c; 1993a; 1994a; 1998a, administration of BBP both by gavage and in the diet) performed studies in which BBP was administered on different days of gestation or in pseudopregnant rats. In most of these studies pair-fed rats were included as reference groups. In rats exposed to BBP on gestation day 7-16 and 11-20, but not in pair-fed rats, an increased incidence of malformations was reported. Furthermore, increased post-implantation loss was observed after exposure on gestation days 0-20, 0-7, 0-8, 0-11, 7-9, 10-12, 13-15 and 7-16 but not after exposure in the later stages of pregnancy (i.e., 16-20 and day 11-20) as compared to control animals and pair-fed rats. In pseudopregnant rats exposed to BBP on day 0-8 of pseudopregnancy uterine decidual growth was decreased at dose levels of 750 mg/kg bw/day and higher, indicating that early embryonic loss may at least in part be mediated via a suppression of uterine decidualisation. These results indicate that the teratogenic effect reported after oral administration of BBP during the organogenic period is primarily the result of BBP exposure, and not a result of the reduced body weight gain observed in dams. BBP or one of its major metabolites are reported to readily cross the placenta barrier, (Kluwe, 1982; Thomas et al., 1986), and is reported in the foetuses (Saillenfait et al., 1998). Maternal toxicity in the studies performed by Ema and co-workers was evaluated in experiments with a long dosing period, but not after a short dosing period. However, maternal toxicity when measured, were present when developmental toxicity was reported.

The effect of low concentration exposure to BBP in drinking water during gestation and early postnatal life on reproductive performance in offspring was evaluated in various rat studies (Sharpe et al., 1995; Ashby et al., 1997; TNO, 1998a and b; Bayer, 1998). A problem with these studies is the instability of BBP in drinking water. The main purpose of the studies was to evaluate a possible estrogenic effect of BBP. However, the results of the studies varied. In the study by Sharpe and coworkers (1995) BBP was shown to affect testicular size and spermatogenesis in offspring after administration of 1,000 µg/l in drinking water corresponding to 0.126 to 0.366 mg/kg bw/day. However, Sharpe and coworkers (1995) considered the biological variance to have a greater influence on the test results than the test compound tested. In a similar (but not identical) study performed by Ashby and coworkers (1997) no effects on testis weight and spermatogenesis were observed in the offspring. Due to the variable results observed in these studies, two TNO studies, (TNO, 1998a and b) were performed which indicated the same results as Ashby and coworkers (1997). However, in these studies there were a high incidence of pup-mortality in both the control animals (higher than historical control data), and in the animals exposed to low concentrations of BBP (0.01 to 0.674 mg/kg/bw/day). No increase in reproductive parameters in BBP exposed animals was reported compared to control animals. A new study was performed to further evaluate pup-mortality (Bayer, 1998). In this study no effects on reproductive parameters were reported after exposure to low concentrations of BBP (0.09 to 0.8 mg/kg/bw/day), and no increase in pup-mortality was reported in the control animals compared to historical control data, or in the BBP exposed animals. Overall, these studies clearly indicate that no impairment of the reproductive system in the offspring are observed in rats exposed to very low concentrations of BBP during the gestation and lactational period.

The developmental effects of the major BBP metabolites were also investigated in several studies by Ema and co-workers, since BBP is readily hydrolysed after oral administration in the gastro-intestinal tract and the liver to the corresponding monophthalate-esters (MBuP and MBeP). In these

studies the developmental toxicity of MBuP and MBeP were evaluated after oral administration in rats on pregnancy day 7-15, or after oral administration of MBeP on pregnancy day 7-9, 10-12, 13-15 or 15-17. The pattern of developmental toxicity observed after exposure to MBuP or MBeP was almost similar to the effects observed after exposure to BBP, suggesting that MBuP and MBeP may be responsible for the embryotoxic and/or teratogenic effect of BBP. In the study bu Imajima et al. (1997) testicular descendent was studied, which is under androgic control. In this study the testis were located significantly higher in the abdominal cavity on gd 20 in rats exposed *in utero* to MBuP (approximately 1,000 mg/kg bw/day) from gd 15-18 compared to control rats, furthermore, on pnd 30-40 cryptorchidism was reported in 84.6% of the exposed offspring, compared to 0% in the control group. Furthermore, Shono et al. (2000) studied the time-specific effects of MBuP on the transabdominal migration of the testis in foetal rats. The foetuses were exposed to MBuP (approximately 1,000 mg/kg bw/day) *in utero* from gd 7-10, 11-14 or 15-18. The study showed that on gd 20 the testis was located significantly higher in the abdominal cavity after exposure on gd 11-14 or 15-18, the effect was more pronounced after exposure on gd 15-18. Furthermore, the testosterone levels were significantly lower in MBuP treated foetuses compared to control foetuses. No information from the Imajima et al. (1997) study or Shono et al. (2000) study was available regarding maternal toxicity. In the study by Ema et al. (2003) *in utero* exposure to MBeP on gd 15-17 was shown to induce a significant decrease in AGD and a significant increase in the incidence of undescended testis.

MBuP is a major metabolite of DBP. In studies where DBP was administered during the organogenesis in rats (Ema et al., 1993b; 1994b; 1998b) malformations in the foetuses were reported. DBP administration during the organogenic and/or late gestation period (Mylchreest et al., 1998) induced a similar pattern of malformations in the male reproductive system as were reported after exposure to BBP (Gray et al., 2000; Parks et al., 1999). However, it should be emphasised that the results of the DBP studies have not been used as the basis for the conclusions and classification proposal for BBP.

The National Toxicology Program (NTP) Center for the evaluation of risk to human reproduction has used a phthalate expert panel to evaluate the reproductive and developmental toxicity of BBP and other phthalates. This expert panel has concluded that the database on developmental toxicity is suffucient to judge that oral exposure to BBP can cause developmental toxicity in rats and mice (Kavlock et al., 2002). Developmental toxicity was reported in rats and mice exposed *in utero* to BBP in the absence of marked maternal toxicity. In a new 2-generation study in rats (Tyl et al., 2004) the NOAEL for developmental effects in offspring was 50 mg/kg bw/day and the NOAEL for maternal toxicity was 250 mg/kg bw/day. In a recent study in rats exposed to BBP *in utero* from gestation day 6 to 20 the NOAEL for developmental effects was 270 mg/kg bw/day, whereas the maternal LOAEL was 580 mg/kg bw/day. Furthermore, exposure to BBP in late gestation (from gd 15-20) was shown to be important in the determination of developmental effects on the reproductive organs in male offspring. In studies performed to determine if periods of exposure to BBP during pregnancy would modify the developmental toxicity of BBP, teratogenic effects reported after oral administration of BBP during the organogenic period were shown to be a result of BBP exposure. Furthermore, a potential anti-androgen-like activity of BBP has been demonstrated in different *in vitro* and *in vivo* studies. It is concluded that BBP affects development, and is proposed classified with T R61 Repro. Cat. 2, according to EU criteria.In the risk characterisation for developmental effects the NOAEL at 50 mg/kg bw/day from a 2-generation study (Tyl et al., 2004) is used based on a dose-related significant reduction in absolute and adjusted AGD in both F1 and F2 offspring from 250 mg/kg bw/day in the absence of maternal toxicity. In the Nagao et al. (2000) study a decerase in absolute AGD was reported at 500 mg/kg bw/day. In this study the adjusted AGD was not analysed. Since birth weight was reduced at 100 mg/kg bw/day in this study, it is unclear whether analysis of adjusted AGD would have shown decreased AGD at 100 mg/kg bw/day.

4.1.2.9.10 Summary developmental studies, BBP, humans

In a study by Swan et al. (2005) an association between maternal exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all *p*-values < 0.05).

In a study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of contaminated milk with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG, LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005), further studies with larger sample size have to be performed before clear conclusions can be drawn from these studies.

4.1.2.9.11 Endocrine activity of BBP and BBP metabolites in vitro and in vivo

Estrogen activity of BBP in vitro

A potential estrogen activity of BBP and the major metabolites MBuP and MBeP have been assessed in both *in vitro* and *in vivo* studies.

BBP was tested in a recombinant yeast screen for estrogenic activity, where the yeast cells expressed the human estrogen receptor hER. The study included assays to investigate the ability of BBP to interact both as an agonist or antagonist. 17β-estradiol was used as positive control and ethanol served as negative control. To determine whether BBP possessed an anti-estrogenic activcity, the natural ligand (17β-estradiol (2.5 . 10-10 M) was added to the medium at a concentration that produced a sub-maximal response (65%). The estrogen, and anti-estrogenic activity of BBP was tested at BBP concentrations from 10^{-8} to 10^{-4} M. The most potent chemical in each screen was assigned a potency of four plus (++++), and the potency of the chemicals was expressed relative to this. BBP showed a very week estrogenic activity (+), and no anti-estrogenic (-) activity. 17β-estradiol showed a high relative potency (++++) in the hER assay (Sohoni and Sumpter, 1998).

The estrogenic activities of several phthalate esters, including BBP, were investigated *in vitro* using estrogen receptor (ER) competitive ligand binding, mammalian- and yeast-based gene expression, and MCF-7 human breast cancer cell proliferation assays. It was concluded that BBP exhibited weak ER-mediated estrogenic activity based on the results in the studies summarised below in **Table 4.29** (Zacharewski et al., 1998).

Estrogen receptor (ER) competitive ligand binding assay, measuring specific binding of [3H]-E2 (17β-estradiol) to the rat (Sprague-Dawley) uterine ER, was used to detect estrogenic activity of

BBP. ER was isolated from rat uterine. The concentrations of BBP and E2 were 1-1000 μM and 0.001-100 nM. The competitor was either (i) unlabelled E2, (ii) BBP or (iii) DMSO solvent alone. Incubations were carried out at 30 oC for 30 min. BBP weakly competed with E2 for the binding to the ER. Unlabelled E2 exhibited an IC50 of 1.3 nM (which is within the range of previously reported IC50 values), whereas the IC50 value for BBP was approximately 36 μM. In this study E2 was approximately 3 .104 more potent than BBP (Zacharewski et al., 1998).

In the second study, BBP-induced reporter gene expression in recombinant receptor/reporter gene assay using MCF-7 human breast cancer cells or HeLa human cervical carcinoma cells transfected with Gal4-human estrogen receptor chimera (Gal4-HEGO) and the Gal4-regulated luciferase reporter gene (17m5-Glob-Luc) were studied. The concentrations used were: 0.1, 1 and 10 μM of BBP and 1 pM to 10 nM E2 as a positive control. BBP was found to significantly induce luciferase activity. 10 μM of BBP induced an increase in luciferase activity of 46 ± 14% in MCF-7 cells, when compared to the 100 ± 27% response induced by 10 nM E2. 10 μM of BBP induced an increase in luciferase activity in transiently transfected MCF-7 cells of 34 ± 16%, as compared to the maximum 100 ± 20% response following treatment with 10 nM E2 (Zacharewski et al., 1998).

BBP was also tested in the yeast estrogen receptor-mediated growth assay. The PL3 *S. cerevisiae* was transformed with HEG0 (human estrogen receptor) for the determination of ER-mediated growth on selective media. The concentrations used were: 10 μM of BBP or 1 nM E2 as control. 10 μM of BBP was able to weakly support ER-mediated growth of PL3 cells (Zacharewski et al., 1998).

The ability of BBP to act as an estrogen and promote estrogen-dependent cell proliferation was examined using the estrogen-dependent MCF-7BUS human breast cancer cell line. Cell proliferation was evaluated as fold-induction growth relative to the DMSO control. Concentrations used were: 0.1, 1 or 10 μM of BBP or 1 pM to 10 nM of E2 as a positive control. 10 μM of BBP showed significant induction of growth relative to 1 nM E2. The consistency of the assay was extremely variable (Zacharewski et al., 1998).

The recombinant yeast screen test was used to assess possible estrogenic activity of BBP. Concentrations used were: 10^{-3} M to $4.8 \cdot 10^{-7}$ M of BBP or 10^{-8} M to $4.8 \cdot 10^{-12}$ M of E2 (positive control). The yeast cells were incubated in medium for up to 13 days. After 6 days of incubation, BBP possessed estrogenic activity in the screen assay, but approximately one millionfold less than E2. This makes BBP considerably less potent than other environmental estrogens such as bisphenol-A and nonylphenol. The response with BBP reached a plateau at approximately 50% of the maximum response achieved with E2. To determine whether BBP is only a partial estrogen agonist, or whether other explanations account for the sub-maximal response observed, a yeast screen containing BBP was incubated for longer time than usual, and the response monitored daily. On day 4 (the usual incubation time for this yeast assay), the BBP response was weak. On day 13 however, the highest concentration of BBP produced the maximal response possible. Thus the potency of BBP increased with time (Harris et al., 1997).

In the same study (Harris et al., 1997) BBP was also tested for estrogenic activity in two estrogen-responsive human breast cancer cell lines, MCF-7 and ZR-75. The concentrations used in the MCF-7 assay were: 10-5 M of BBP and 10-8 M of E2 as a positive control. Cells were exposed for up to

12 days. For the ZR-75 cells the treatment was: 10^{-5} M, 10^{-6} M, and 10^{-7} M for BBP and 10^{-8} M, 10^{-10} M, and 10-12 M for E2. BBP exhibited estrogenic activity in these assays. Cells were counted at a single end point on day 11. At a concentration of 10^{-5} M, BBP was approximately as potent as 10^{-10} M of E2 in the ZR-75 cells (Harris et al., 1997).

Proliferative potency of BBP was tested in the human breast cancer cells ZR-75. The cells were exposed to 10^{-5} M of BBP and to 10^{-9} M of E2 as a positive control. Cell densities were counted on days 0, 3, 6, 8 and 10. BBP was found to have a potent effect on cell growth at 10^{-5} M, although the growth response was less than the maximal response shown by E2. In the same study, the stimulatory effect of BBP on the transcriptional activity of the estrogen receptor directly was examined on transiently transfected MCF7 cells using the reporter plasmids pTKLUC and pERE-TKLUC. BBP stimulated transcription at concentrations in the range 10^{-6} to 10^{-4} M. At a concentration of 10^{-5} M BBP stimulated transcription of the reporter genes to a similar extent as 10-11 M of E2 (Jobling et al., 1995).

The estrogenic activity of BBP was assessed in the E-SCREEN Test, which measures the growth of human breast MCF-7 cells. Cells were exposed for 6 days, to a range of concentrations of BBP and E2 (concentrations not given). Relative proliferative potencies (RPP, %) were determined, which measures: (the ratio between the minimal concentration of E2 needed for maximal cell yield and the minimal dose of BBP needed to achieve a similar effect) . 100. E2 induced maximal cell yields at 30 pM, whereas BBP was needed at a concentration of 10 µM for maximal cell yield. RPP for BBP was 0.0003% as compared to 100% for E2. BBP was thus weakly positive in the E-SCREEN test (Soto et al., 1995).

The recombinant yeast screen was used to study if DBP or E2 had a synergistic effect on the estrogenic potential of BBP (10^{-4} M or 10^{-5} M BBP; 10^{-4} M or 10^{-5} M DBP; or 10^{-11} M E2). The concentration of E2 used produced only a small response above background, so that, if additive effects or synergism occurred, they could be observed within the range of the assay. In all cases, the response obtained was very close to what was expected if an additive effect had occurred, i.e. no evidence of synergism was observed (Harris et al., 1997).

Estrogen activity of BBP metabolites in vitro

The recombinant yeast screen, in which the human estrogen receptor has been integrated in a form capable of binding to estrogen response elements, and controlling the expression of the reporter gene lac-Z was used to assess the estrogenic potential of MBuP and MBeP from 10^{-3} M to $4.8 \cdot 10^{-7}$ M. None of the metabolites exhibited estrogenic activity in this assay (Harris et al., 1997).

Estrogen activity of BBP in vivo

The potential of BBP (purity > 98.5%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered BBP at dose levels of 0, 56, 280, 1,120, and 2,240 mg/kg bw by gavage. 2,240 mg/kg was the maximum tolerated dose. All doses were administered daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg of oestradiol benzoate/kg bw served as a positive control. Following each daily dose, animals at the two highest dose levels remained subdued for several hours, but had recovered before the next dosing. Mean body weight gain was significantly reduced in the high dose group. There were statistically significant reductions in both absolute and relative uterine weights in the 1,120 mg/kg/day group (79%, $p < 0.05$ and 81%, $p < 0.05$). No reduction in

uterine weight was noted in the high dose group. The positive control increased the absolute uterine weight 3.44-fold ($p < 0.001$) and the uterine body weight ratio was increased 3.67-fold ($p < 0.001$). The report concluded that BBP does not possess the potential to promote uterine growth in immature female rats when dosed orally (Monsanto, 1996b).

The potential of BBP (purity > 98.5%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following subcutaneous exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered BBP at dose levels of 0, 0.5, 5, 50, 500 and 5,000 mg/kg bw by subcutaneous injections. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate was used as a positive control. There were no treatment-related effects or clinical signs in the BBP treatment groups. There were statistically significant reductions in both absolute and relative uterine weights in the 5 mg/kg bw/day BBP group (76% and 77%). None of the other BBP groups were affected. Absolute uterine weight in the positive control group increased 3.91-fold ($p < 0.01$) and 4.00-fold based on the uterine:body weight ratio. The report concluded that BBP does not possess the potential to promote uterine growth in immature female rats when dosed subcutaneously (Monsanto, 1996a).

BBP was tested for its ability to induce increased uterine wet weight (animal weight 50-55 g) and vaginal cell cornification (animal weight 175-200 g) in OXV Sprague-Dawley rats. The rats were dosed by gavage, once daily for a period of 4 days. The dose levels were 0, 20, 200, and 2,000 mg BBP/kg bw/day. 1 mg/kg bw/day of ethynyl oestradiol (EE) was used as positive control. In the uterine and cell vaginal cornification assay animals (groups of 10) were killed on day 5. EE exposure resulted in a 7-fold increase in uterine wet weight. No significant increase in uterine weight was noted with BBP. Oral treatment with EE induced vaginal cell cornification in all animals by day 3 and this was sustained through days 4 and 5 as determined by vaginal smears. 1 mg/kg bw/day EE was scored as 100% efficacious in inducing the keratinisation of vaginal cells. The results with BBP were 3%, 0% and 0% for 20, 200 and 2,000 mg/kg bw/day of BBP. The numbers of positive smears were: control 0/10, positive control 10/10, and for BBP 20 mg/kg bw/day 2/10, 200 mg/kg bw/day 0/10 and 2,000 mg/kg bw/day 0/9 (Zacharewski et al., 1998).

BBP was tested for its ability to induce increased uterine vascular permeability in female Swiss albino ovariectomized mice (3 month of age) 4 hours after a single subcutaneous (sc) administration of 10-4 mol BBP in 0.1 ml saline. The permeability of the uterine vasculature was measured from the leakage of intravenously administered [125I]-labelled human serum albumin. In this acute *in vivo* assay BBP produced no significant effect on uterine vascular permeability (Mulligan et al., 1998).

The effect of BBP on gene expression in the adult hypothalamus of female rats was studied by examining the effect of BBP and 17β-estradiol (positive control) on the expression of estrogenregulated mRNAs, i.e. progesterone receptor (PR) mRNA, preproenkephalin (PPE) mRNA, and neurotensin (NT) mRNA, in the hypothalamus and pituitary of adult female Wistar rats. Female rats 7-8 weeks of age were ovariectomised (OVX).Two weeks after OVX the rats were subcutaneously injected with 10 mg BBP or 10 µg 17β-estradiol in sesame oil, or with sesame oil alone as negative control. Twenty four hours after injection, tissues including the preoptic area (POA) mediobasal hypothalamus (MBH) and anterior pituitary were collected. Nothern blot revealed that injection of 17β-estradiol resulted in expected changes, i.e. significant increase in PR mRNA in the POA, MBH and anterior pituitary, and in PPE mRNA in the MBH. Injection of BBP

increased PR mRNA in the POA and anterior pituitary, although the increase in the anterior pituitary was not significant. BBP failed to induce changes in either NT mRNA in the POA or PPE mRNA in the MBH. The explanation given by the authors was that the estrogenic activity of BBP is weak, and thus revealed only on genes strongly regulated by estrogens, or in tissues highly sensitive to estrogens (Funabashi et al., 2001).

Estrogen activity of BBP metabolites in vivo

The major metabolites of BBP are monobutyl phthalate (MBuP) and monobenzyl phthalate (MBeP). Larger quantities of MBuP than MBeP are formed from BBP (44% MBuP versus 16% MBeP) (Eigenberg et al., 1986; Mikuriya et al., 1988). In this section the estrogen activity of MBuP and MBeP *in vivo* are presented. The potential of MBuP (purity > 99%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered MBuP at dose levels of 0, 1, 10, 100 and 1,000 mg/kg bw by gavage. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate served as a positive control. There were no treatment-related effects or clinical signs in MBuP exposed rats and no significant differences in the group mean terminal body weights or body weight gains. MBuP caused no significant effects on either the absolute uterine weight or relative uterine:body weight ratio. In the positive control group the absolute uterine weight was increased 3.91-fold ($p < 0.01$) and the uterine:body weight ratio was increased 4.00-fold ($p < 0.01$) (Monsanto, 1996c).

The potential of monobenzyl phthalate (MBeP; purity > 99%) to promote uterine growth in immature (20-22 days old) female Alkp:APfSD rats following oral exposure was evaluated using a standard *in vivo* uterotrophic assay. Groups of six rats were administered MBeP at dose levels of 0, 50, 250, 500, 1,000 and 1,500 mg/kg bw by gavage. All doses were given daily for three days and animals were killed 24 hours after the final dose. The uteri were excised and weighed. 0.01 mg/kg bw/day of oestradiol benzoate served as a positive control. After a single dose of MBeP, animals in the 1,000 and 1,500 mg/kg groups became subdued and exhibited piloerection. The severity of these effects was dose-dependent. One animal in the 1,500 mg/kg group died and another animal in this group remained subdued. Rest of the animals in this group was killed. Post-mortem examination of the animals killed prior to scheduled termination revealed distension of the bladder. In two of the remaining animals, the kidneys were slightly pale in colour. MBeP caused a significant decrease in absolute uterine weight in both the 500 and 1,000 mg/kg bw/day groups (79% and 69%). Statistically significant reduction in relative uterine weight was also noted for the 500 and 1,000 mg/kg bw/day MBeP groups (80% and 71%). No significant effects on uterine weight were observed in the 50 or 250 mg/k bw/day treatment groups. In the positive control group the absolute uterine weight was increased 3.44-fold ($p < 0.01$) and the uterine:body weight ratio was increased 3.67-fold ($p < 0.01$) (Monsanto, 1996d).

Summary estrogen activity of BBP and BBP metabolites in vitro and in vivo

A potential estrogen activity of BBP and the major BBP metabolites MBuP and MBeP have been assessed in both *in vitro* and *in vivo* studies. The main results from the various studies are given in **Table 4.29**.

Study Design	Critical Effect	Reference
***In vitro*, BBP**		
ER competetive ligand binding assay; 1-1,000 µM BBP; 30 minutes incubation.	BBP weakly competed with E2 for binding to ER. E2 was approx. 3 - 10^4 more potent than BBP.	Zacharewski et al. (1998)
Gene expression in recombinant receptor/reporter (luciferase) gene assay; 0.1, 1, 10 µM BBP.	10 µM BBP increased reporter gen (luciferase) activity.	Zacharewski et al. (1998)
ER-mediated growth of yeast assay; 10 µM.	10 µM of BBP weakly supported ER-mediated growth of yeast cells.	Zacharewski et al. (1998)
Estrogen-dependent cell proliferation assay; 0.1, 1 and 10 µM BBP.	10 µM showed induction of cell growth.	Zacharewski et al. (1998)
Gene expression in recombinant receptor/reporter (ß-galactosidase) gene assay; 13 days incubation; 48-1,000 µM BBP.	After 6 days BBP possessed weak estrogenic activity. The activity was one millionfold less than E2.	Harris et al. (1997)
Estrogen-dependent cell proliferation assay; 12 days incubation; 10 µM BBP.	10 µM BBP exhibited estrogenic activity measured as induced cell growth. E2 was approx. 10^4 more potent than BBP.	Harris et al. (1997)
Estrogen-dependent cell proliferation assay (E-SCREEN); 6 days.	10 µM BBP was needed for maximal cell yield, whereas 0.00003 µM of E2 was needed.	Soto et al. (1995)
Estrogen-dependent cell proliferation assay; 100 µM BBP.	A potent effect on cell growth was reported at 10^{-5} M BBP, however this effect was less potent than exposure to 10^{-9} M E2.	Jobling et al. (1995)
Transcriptional activity of ER directly; 0.01-1 µM BBP.	10^{-5} M (0.1 µM) BBP stimulated transcription to similar extent as 10^{-11} M E2.	Jobling et al. (1995)
Yeast cells expressing human estrogen or androgen receptor, agonistic or antagonistic properties studied; BBP cons. from 10^{-8} to 10^{-4} M	BBP was a weak estrogen, negativ androgen and anti-estrogen and a potent anti-androgen.	Sohoni and Sumpter (1998)
***In vitro* BBP metabolites, MBuP and MbeP**		
MBuP and MBeP; Gene expression in recombinant receptor/reporter (ß-galactosidase) gene assay; 48 – 1,000 µM.	No estrogenic activity of MBuP and MBeP.	Harris et al. (1997)

Study Design	Critical Effect	Reference
***In vivo*, BBP**		
Alkp:ApfSD immature rats; 6 female/group; gavage; 3 days; 56, 280, 1,120 and 2,240 mg/kg bw/day BBP; standard *in vivo* uterotrophic assay.	At 1,120 but not at 2,240 mg/kg bw/day, reduced absolute and relative uterine weight. At 2,240 reduced body weight.	Monsanto (1996b)
Alkp:ApfSD immature rats; 6 female/group/ subcutaneous administration; 3 days; 0.5, 5, 50, 500 and 5,000 mg/kg bw/day BBP; standard *in vivo* uterotrophic assay.	At 5 mg/kg bw/day, but not at the higher doses, reduced absolute and relative uterine weight.	Monsanto (1996a)
Swiss albino ovariectomized mice (3 month); subcutaneous injection in 0.1 ml saline; 4 hours; 10^{-4} mol BBP.	No effect on uterine vascular permeability.	Mulligan et al. (1998)
OXV Sprague-Dawley rats; 10 female/group by gavage; 4 days; 20, 200 and 2,000 mg/kg bw/day; vaginal cell cornification assay.	No increase in uterine weight.	Zacharewski et al. (1998)
OVX Wistar rats; one subcutaneous injection; 10 mg BBP or 10 µg 17β-estradiol (positive control). The expression of estrogen regulated mRNAs were studied i.e. progesterone receptor (PR) mRNA, preproenkephalin (PPE) mRNA, and neurotensin (NT) mRNA, in the hypothalamus and pituitary	17β-estradiol resulted in expected changes, i.e. significant increase in PR mRNA in the preoptic area (POA), mediobasal hypothalamus (MBH) and anterior pituitary, and in PPE mRNA in the MBH. Injection of BBP increased PR mRNA in the POA and anterior pituitary, although the increase in the anterior pituitary was not significant. BBP failed to induce changes in either NT mRNA in the POA or PPE mRNA in the MBH.	Funabashi et al. (2001)
***In vivo* BBP metabolites, MBuP and MbeP**		
MBuP; Alkp:ApfSD rats; 6 female/group; gavage; 3 days; 1, 10, 100 and 1,000 mg/kg bw/day; standard *in vivo* uterotrophic assay.	No increase in uterine weight.	Monsanto (1996c)
MBeP; Alkp:ApfSD rats; 6 female/group; gavage; 50, 250 500, 1,000 and 1,500 mg/kg bw/day; standard *in vivo* uterotrophic assay.	At 500 and 1,000 mg/kg bw/day reduced relative and absolute uterine weight. At 1,000 and 1,500 mg/kg bw/day piloerection.	Monsanto (1996d)

In vitro

The *in vitro* studies includes a recombinant yeast screen assay, an estrogen-receptor (ER) competitive ligand binding assay, mammalian- and yeast-based gene expression assays and an estrogen-dependent cell proliferation assay. In these assays performed to evaluate a possible estrogen activity of BBP, only a weak estrogen activity at high concentrations of BBP (10-100 µM) was reported. In the same assays E2 (17ß-estradiol) was approximately 104 to 106 more potent than BBP. The metabolites MBuP and MBeP did not exhibit estrogenic activity in a recombinant yeast screen assay.

In vivo

The estrogenic activity of BBP and its major metabolites were studied in standard *in vivo* uterotrophic assays. In these studies BBP and MBuP did not possess the potential to promote uterine growth in immature female rats exposed orally to BBP (up to 2,240 mg/kg bw/day) or subcutaneous to BBP (up to 5,000 mg/kg bw/day), whereas, MBeP caused a significant reduction in absolute and relative uterine weight at 500 and 1,000 mg/kg bw/day, however, at 1,000 mg/kg bw/day the animals became subdued, and at 1500 mg/kg bw/day the animals were killed prior to

scheduled termination due to systemic toxicity. From the developmental study with MBeP (Ema et al., 1995a) it seems like MBeP is more toxic than BBP (reduced weight gain in dams, reddis stainig of the facial fur, pilo-erection, and spasticity from 313 mg/kg bw/day). Taking these considerations into account, and that no reduction in uterin weight in three other studies (Zacharewski et al., 1998 where both Alpk:ApfSD immature rats and OXV Sprague-Dawley rats were studied and Monsanto 1996c), and that BBP only possessed a very week estrogenic ativity in various *in vitro* studies, the reduction in uterine weight reported in the Monsanto (1996d) study is considered to be of limited importance in the evaluation of a potential estrogenic activity of BBP *in vivo*. The results from the *in vivo* uterotrophic assays are in accordance with the results reported in the low dose drinking water studies (TNO, 1998a and b; Bayer, 1998; Ashby et al., 1997; see developmental section). In these studies no effects on reproductive parameters were reported after exposure to very low concentrations of BBP (0.01-0.674 mg/kg bw/day) *in utero* and during lactation.

In a study by Funabashi et al. (2001) the expression of estrogen regulated mRNAs was studied in the hypothalamus, preoptic area and pituitary in OVX female rats following subcutaneous injection of 10 mg BBP or 10 μg 17β-estradiol (positive control). 17β-estradiol resulted in expected changes in mRNAs. However, injection of BBP increased PR mRNA only in the preoptic area. It was concluded that that the estrogenic activity of BBP was weak, and thus revealed only on genes strongly regulated by estrogens, or in tissues highly sensitive to estrogens.

Anti-androgen activity of BBP in vitro

BBP was tested in a recombinant yeast screen for androgen activity where the yeast cells expressed the human androgen receptor hAR. The study included assays to investigate the ability of BBP to interact both as an agonist or antagonist. Dihydrotestosterone (DHT) was used as a positive control and ethanol served as negative control. To determine whether BBP possessed an anti-androgen activcity, DHT (1.25 . 10-9 M) was added to the medium at a concentration that produced a sub-maximal response (65%). The androgen and anti-androgenic activity of BBP was tested at BBP concentrations from 10-8 to 10-4 M. The most potent chemical in each screen was assigned a potency of four plus (++++), and the potency of the chemicals were expressed relative to this. BBP showed no androgen activity (-), however, was a potent anti-androgen (++++). BBP was as potent as the known anti-androgen flutamide. DHT had a high relative potency (++++) in the hAR assay (Sohoni and Sumpter, 1998).

Anti-androgen activity of BBP, MBuP and MBeP in vivo

In this section the nine recent studies (Tyl et al., 2004; Piersma et a., 2000; Gray et al., 2000; Parks et al., 1999; Imajima et al., 1997; Nagao et al., 2000; Shono et al., 2000; Ema et al., 2002; Ema et al., 2003) are presented since effects, which may be indicative of an anti-androgen-like activity of BBP, MBuP or MBeP were reported. These effects includes reduced testicular weight, reduced ano-genital distance (AGD), and retarded transdominal descent of testis in male offspring exposed to BBP, MBuP or MBeP during the organogenic period and/or the late prenatal early postnatal period. Furthermore, this section is also supplemented with the conclusions from the studies where an anti-androgen-like effect of DBP is proposed by the authors (Mylchreest et al., 1998; Ema et al., 1998b), since one of the major metabolites of DBP is MBuP (Albro and Moore, 1974; Williams and Blanchfield, 1975; Tanaka et al., 1978). Furthermore, the pattern of malformations reported in foetuses after exposure to MBuP and MBeP were similar (Ema et al., 1995; Ema et al., 2003) to that produced by BBP (Ema et al., 1992a,b,c) and DBP (Ema et al., 1993b; 1994b).

In the Piersma et al. (2000) developmental toxicity study pregnant rats were exposed to BBP by gavage (0, 270, 350, 450, 580, 750, 970, 1,250, 1,600 or 2,100 mg/kg/day) *in utero* from gestation day (gd) 6 to 15 (short time exposure) or 6 to 20 (long time exposure) and necropsied on post-natal day 21. BBP induced reduced testicular weight in offspring exposed to BBP from gd 6-20 with a LOAEL of 270 mg/kg/day. Retarded transdominal descent of testis was reported with a LOAEL of 580 mg/kg/day. This effect was more pronounced after long time exposure *in utero* to BBP. The maternal LOAEL was 580 mg/kg/day based on increased liver weight. For more study-information see Section 4.1.2.9.

In the Gray et al. (2000) and Parks et al. (1999) studies pregnant Sprague-Dawley rats were given 750 mg/kg/day of BBP by gavage from gestation day (gd) 14 through postnatal day (pnd) 3. Reduced AGD and testis weight was reported on pnd 2, and areolas on pnd 13 were seen in the male offspring in both studies. In the Gray et al. (2000) study male offspring at approximately 90 days of age exposed from gd 14 through pnd 3 was also studied for malformations in the reproductive organs, and 84% of the male offspring were reported to have malformations in the testis, accessory reproductive organs and genitalia. For more studyinformation see Section 4.1.2.9.

In the Imajima et al. (1997) study the effect of prenatally exposure to Monobutyl phthalate (MBuP) approximately 1,000 mg/kg bw/day on testicular descent was studied in Wistar-King A rats. Pregnant rats were gavaged from gestation day 15 to 18. In the control offspring all the testes were located in the lower abdominal cavity near the bladder neck on gd 20, whereas, in offspring treated *in utero* to MBuP the testes were located significantly higher in the abdominal cavity, and some were located near the kidney. On pnd 30 – 40, in the control group all testes descended into the scrotum and the incidence of cryptorchidism was 0%, whereas, in the MBuP treated offspring 22 rats showed cryptorchidism (14 unilateral and 8 bilateral undescended testes), and the incidence of cryptorchidism was 84.6%. This may indicate that MBuP act in an anti-androgenic manner, since testis descent is under androgenic control. For further study description see Section 4.1.2.9.

In the Shono et al. (2000) study the time-specific effects of monobutyl phthalate (MBuP) on the transabdominal migration of the testis, and the levels of testosterone were studied in foetal rats. Three groups of pregnant Wistar-King A rats were administered MBuP by gavage (2-6 pregnant rats/group, 0.3 g/day, corresponding to approximately 1,000 mg/kg bw/day). Group 1 was exposed from gestation day (gd) 7-10, group 2 from gd 11-14, and group 3 from gd 15-18. The control group (group 4) received vehicle from gd 7-18. At gd 20 the foetuses were obtained by Caesarean section, and the position of the testes were determined in all groups. The results from this study showed that in the control group all 30 testes were anchored at the bottom of the abdominal cavity near the bladder neck by a swollen gubernaculum, whereas in group 3 (exposure to MBuP from gd 15-18) the testes were high in the abdominal cavity and associated with both an elongated gubernaculum and a hypertrophic cranial suspensory ligament. The mean transabdominal testicular migration values (the distance from the bladder neck to the lower pole of the testis) in group 1, 2, 3, and 4 were 12.3 ± 5.9 (10 testes), 24.5 ± 5.2 (10 testes), 57.9 ± 2.6 (38 testes), and 9.3 ± 1.9 (30 testes). Values were significantly higher in group 2 and 3 compared to the control group. The mean testosterone levels were 50.9 ± 3.8 pg/testis in MBuP treated foetuses (25 testes) and 852 ± 80.3 pg/testis in the control foetuses (30 testis), the levels was significantly lower in the MBuP treated rats compared to the controls. For further study description see Section 4.1.2.9.

Ema et al. (2002) studied he effects of BBP on the development of the reproductive system in male offspring. In this study pregnant Wistar rats (16/group) were given BBP by gastric intubation at doses of 250, 500 or 1,000 mg/kg on days 15 to 17 of pregnancy. A significant increase in the incidece of foetuses/litter with undescended testes was found at 500 (54/14) and 1,000 (97/16)

mg/kg compared to 0/16 in the control group. Furthermore, a statistically significant decrease in the AGD of male foetuses was observed at 500 and 1,000 mg/kg. The AGD/cube root of body weight ratio in male foetuses was also significantly reduced from 500 mg/kg. The AGD/cube root of body weight ratio in female foetuses in the BBP treated groups were comparable to those in the control group. It was concluded by the authors of the study that BBP given to pregnant rats during gestation day 15-17 produced adverse effects on the development of the reproductive system in male offspring. For further study description see Section 4.1.2.9.

Ema et al. (2003) studied the effect of monobenzyl phthalate MBeP, a major metabolite of BBP on the development of the reproductive system in rats. He also looked into the role of MBeP in the antiadrogenic effects of BBP. In this study pregnant Wistar rats (16/dose group) were given MBeP by gavage at doses of 167, 250 and 375 mg/kg bw/day on gestation day (gd) 15 to 17. Foetuses were examined on gd 21. Maternal body weight gain on gd 15-18 was significantly decreased from 167 mg/kg bw/day (31, 24, 23 and 15g in the control, 167, 250 and 375 mg/kg bw/day dose group). Maternal food consumption was significantly decreased on gd 15-18 from 167 mg/kg bw/day (54, 46, 40 and 33 in the control, 167, 250 and 375 mg/kg bw/day dose group). The adjusted maternal weight gain was significantly decreased from 250 mg/kg bw/day. Foetal weight was significantly decreased at 375 mg/kg bw/day. A significant increase in the incidence of undescended testes/litter was reported from 250 mg/kg bw/day (2(2), 1(1), 21(12) and 79(16) in the control, 167, 250 and 375 mg/kg bw/day dose group). A decrease in the AGD and ratio of AGD on the cube root of body weight was reported in male foetuses at 250 mg/kg bw/day. No effect on AGD was found in female foetuses. The study indicated that MBeP produced adverse effects on the development of the reproductive system in male offspring and suggested that MBeP may be responsible for the antiandrogenic effects of BBP.

In the 2-generation study (Tyl et al., 2004) a dose related significant reduction in the absolute and adjusted AGD was reported in the F1 and F2 pups from 250 mg/kg bw/day. Reduced AGD at birth is reported to be one of the most sensitive indicators of andorgenic activity (Gray et al., 1997). Furthermore, at 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae were reported. At weanling in F1 and F2 offspring a significant reduction in testis weight was reported in the 750 mg/kg bw/day dose group. At post natal day 21 necropsies the percentage of males with reproductive tract malformations (RTM) were significantly increased at 750 mg/kg bw/day in the F1 and F2 offsprings, and at adult necropsies the percentage of males with RTM were significantly increased in the F1 offspring (F2 offspring was not evaluated as adults). In F1 parental male a significant decrease in the testis, epididymis, prostate and seminal vesicle weights were reported (not evaluated in the F2 generation).

In the two-generation study performed by Nagao et al. (2000) a decrease in the weights of testis, epididymis, and seminal vesicle were reported in the F1 generation exposed to 500 mg/kg bw/day BBP *in utero* or via milk, when evaluated at weaning or after puberty. In addition, in the same group, tubular atrophy and decreased germial epithelium was observed. A decrease in AGD was reported in male F1 offspring. For further study description see Section 4.1.2.9. The effects of DBP on prenatal and early neonatal development of the reproductive tract in rats were studied *in vivo* (Mylchreest et al., 1998). In this study a marked disturbed development of the male reproductive tract (internal and external) in rat offspring exposed via their mothers during gestation and lactation was observed at all dose levels (250, 500 or 750 mg/kg bw/day by gavage) in the absence of significant maternal toxicity. In female offspring sporadic cases of reproductive tract malformations were observed at 500 and 750 mg/kg bw/day. Age at vaginal opening and estrus cyclicity was not affected. The results of this study suggested that DBP does not possess estrogenic activity but rather shows anti-androgenic activity at these dose levels. The results reported in the Mylchreest et al.

(1998) study were confirmed in a study by Ema et al. (1998b). In this study Wistar rats were exposed to DBP (331, 555 or 661 mg/kg bw) from gestation day 11-21.

Summary anti-androgen activity of BBP in vitro and in vivo

BBP was shown in one *in vitro* study to be a potent anti-androgen in yeast cells expressing the androgen receptor. Nine *in vivo* studies are available which indicate an anti-androgen-like activity of BBP or its major metabolites in rats, MBuP and MBeP (Piersma et al., 2000; Gray et al., 2000; Parks et al., 1999; Imajima et al., 1997; Shono et al., 2000; Nagao et al., 2000; Tyl et al., 2004; Ema et al., 2002; Ema et al., 2003). Effects reported in the Piersma et al. (2000) study included a reduction in testicular weight in offspring, and effects on testicular migration from 270 mg/kg bw/day and 580 mg/kg bw/day after *in utero* exposure to BBP from gestation day 6 to 20. In the Gray et al. (2000) study malformations in the reproductive organs in 84% of male offspring (approximately 90 days of age) exposed to 750 mg/kg bw/day BBP from gestation day 14 through postnatal day 2 were reported. Furthermore in the Gray et al. (2000) and Parks et al. (1999) studies a reduced AGD and testis weight in males at day 2 of age, and males with areolas at day 13 of age were reported. In the Imajima et al. (1997) study and the Shono et al. (2000) study testicular descendent was studied, which is under androgenic control. In this study the testis were located significantly higher in the abdominal cavity on gd 20 offspring compared to control rats exposed *in utero* to MBuP from gd 15-18. Furthermore, in the Imajima et al. (1997) study, on pnd 30-40 cryptorchidism was reported in 84.6% of the exposed offspring, compared to 0% in the control group. In the study by Ema et al. (2002) *in utero* exposure to 500 and 1,000 mg/kg BBP on gd 15-17 induced a significant decrease in the AGD and a significant increase in the incidence of undescended testis. In the study by Ema et al. (2003) *in utero* exposure to MBeP on gd 15-17 was shown to induce a significant decrease in AGD and a significant increase in the incidence of undescended testis. In the study by Nagao et al. (2000) a decrease in the weight of the testis, epididymis, and seminal vesicle, and tubular atrophy and decreased germinal epithelium was reported in F1 male offspring exposed to 500 mg/kg bw/day BBP during gestation and lactation and evaluated at weaning or after puberty. Furthermore, a decrease in AGD was reported in male offspring in the 500 mg/kg bw/day dose group, which is a sensitive indicator of anti-androgen activity. In the Tyl et al. (2004) study a dose-related decrease in absolute and adjusted AGD was reported in F1 and F2 male pups from 250 mg/kg bw/day. Furthermore, at 750 mg/kg bw/day in F1 and F2 offspring a significant decrease in reproductive organ weights, and a significant increase in the percentage of males with reproductive tract malformations were reported. A potential anti-androgen-like effect of DBP has been indicated in different studies (Mylchreest et al., 1998; Ema et al., 1998b; Gray et al., 1998; Foster et al., 1998). In some of these studies the authors proposed that the major metabolite of DBP; MBuP may elicit an anti-androgen-like effect.

An association between prenatal and postnatal exposure to phthalates and whether the exposure had any influence on reproductive organ development in newborn boys was studied in two epidemiological studies. In the study by Swan et al. (2005) an association between maternal exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all p-values < 0.05).

In the study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of milk contaminated with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG,

LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005) further studies with larger sample size have to be performed before clear conclusions can be drawn from these studies.

4.1.3 Risk characterisation

4.1.3.1 General aspects

The human population may be exposed to BBP at the workplace, from the use of consumer products, and indirectly via the environment (see Section 4.1.1.2, 4.1.1.3, and 4.1.1.4). The main exposure routs for wokers are expected to be inhalation and dermal contact. Ingestion is considered not to be relevant for occupational exposure. For consumers, and humans exposed indirectly via the environment, the main exposure is expected to be from ingestion. In recent studies urinary phthalate metabolites were measured in human reference populations. These studies indicated that human exposure to phthalates including BBP is both higher and more common than previously suspected (Blount et al., 2000a; CDC, 2001; CDC, 2003; Hoppin et al., 2002; Koch et al., 2003; Brock et al., 2002; Adibi et al., 2003). See Section 4.1.1.4 for study description.

In rats, the kinetics of BBP after oral administration was dose-dependent. Excretion of radiolabelled BBP in the urine was between 70% and 80% in the dose-range of 2 mg/kg p.o. and 200 mg/kg p.o. whereas 22.4% were excreted in the urine after administration of 2,000 mg/kg p.o. The excretion of radioactivity in the feces was 20% after intravenous administration which indicates that the absorption in the dose range between 2 mg/kg p.o. and 200 mg/kg p.o. is nearly complete. After dermal application, 30-40% of the applied amount seems to be absorbed and reaches the systemic circulation. The extent of systemic availability of the substance administered by inhalation is not known as specific data are lacking.

BBP is metabolized to monobutyl phthalate (MBuP) or monobenzyl phthalate (MBeP). This metabolism may take place in the gut wall and/or liver. In adult and immature rats, the ratio of monobutyl phthalate to monobenzyl phthalate found in the urine is 3:1. Both metabolites were found in the bile. Reabsorption from gut lumen may take place. There is no evidence of tissue accumulation. The percentage of excreted metabolites (MBuP and MBeP) in the urine in adult rats was shown to be higher compared to immature rats. The excretion of BBP metabolites in urine has also been studied in humans. Contrary to the metabolism of BBP in rats, BBP is mainly metabolised to MBeP in humans. However, limited data on the metabolism of BBP in humans is available.

No half-life of BBP in the body has been calculated. However, the available data indicate a halflife of less than 24 hours.

In the risk characterisation, 100% absorption is assumed for both inhalation and oral exposure, whereas the absorption for dermal exposure is set at 5%.

None of the acute toxicity studies have been performed according to current guidelines or in compliance with GLP. The acute toxicity of BBP in animals is low. The oral LD50 values of BBP ranged from 2,330 – 20,400 mg/kg bw/day in rats and was 4,170 mg/kg bw/day (female) and 6,160 mg/kg bw/day (male) in mice. The dermal LD50 value in rabbits was greater than 10,000 mg/kg bw/day, whereas in rats the dermal LD50 value was 6700 mg/kg bw/day. The LD50 values of BBP

from i.p. administration were in the same range as from oral or dermal exposure. No information on acute toxicity after inhalation exposure is identified. The wide range of oral LD50 values in rats may be due to the water insolubility of BBP. The lowest LD50 value was obtained when BBP was administered in a corn oil vehicle.

With respect to the irritation potential of BBP, animal studies performed according to current standards for both skin and eye irritation were available, whereas in humans only skin irritation was studied. From these studies it appears that BBP is not irritating to the skin, however, a slight eye irritation was reported in rabbits using the Draize procedure. No data on respiratory irritation from animal or human studies are available.

As regards the sensitizing effect of BBP both animal and human studies were located. In an ear swelling test in mice and guinea pigs, BBP was negative. However, the test has not been fully evaluated and no standard protocols are available. In two human studies no sensitisation of BBP was reported. No data on respiratory sensitisation from animal studies are available. In a casecontrol study an association was found between childeren exposed to BBP in house dust and cases of allergic symptoms. However, in this study very small differences were found in the concentrations of BBP in house dust from controls and cases of allergic symptoms in children, and the children were exposed to other phthalates (DBP, DEHP etc) as well. Furthermore, demographic factors and pet ownership were not considered in this study. Due to the limitations in study design, no clear conclusion can be drawn from the study on the relationship between BBP in house dust and allergic symptoms in children.

With respect to repeated dose toxicity, the data from a well performed 13 week study with oral administration of BBP to rats revealed a NOAEL of 151 mg/kg bw/day (Hammond et al., 1987). This NOAEL value is used in the risk assessment for consumers and indirect exposure via the environment for oral exposure to BBP. A 13 week inhalation study in rats performed in compliance with GLP revealed a NOAEL of 218 mg/m3 (Monsanto, 1982). This NOAEL value is used in the risk assessment for workers for inhalation exposure to BBP, and for indoor air exposure to BBP for consumers. In the oral repeated dose toxicity study histopathological changes, gross morphological changes, and increased kidney weight and an urinary pH decrease were reported at the next highest BBP dose; 381 mg/kg bw/day in male rats. In the inhalation repeated dose toxicity study a significantly increased kidney and liver weight was reported at 789 mg/m3 in male and female rats, and a decrease in serum glucose in male rats.

Based on the data available for BBP from a variety of *in vitro* and *in vivo* genotoxicity studies; mutagenicity in *Salmonellea typhimurium* or in mouse lymphoma cells; sister chromatid exchanges (SCE) or chromosomal abberations (CA) in CHO hamster cells; morphological transformation in Syrian hamster embryo cells or BALB/3T3 cells; sex-linked recessive lethals in *Drosophila melanogaster* or dominant lethal mutations in mice, and taking into consideration the non-genotoxic properties of other phthalate esters, BBP can be considered as a non-genotoxic substance.

Phthalate esters are known to induce peroxisom proliferation in the liver of mice and rats. In general the longer chain dialkylphthalates are more potent inducers than the shorter chains, and branched chain phthalates seemed more potent than straight. Many peroxisome proliferators have been shown to induce hepatocellular tumours when administrated at high dose-levels for long periods to mice and rats despite being non-genotoxic. The mechanisms of induction of carcinogenicity by peroxisome proliferators are considered to have a threshold. Species differences in sensitivity to chemicals that induce peroxisome proliferation are reported. Mice and rats are very sensitive, hamsters have a less marked response, whereas guinea-pigs, primates and humans are rather insensitive or non-responsive. BBP induce peroxisome proliferation in rats, however compared with

Di-(ethylhexyl) phthalate BBP appears to be less effective in causing peroxisome proliferation. As regards the carcinogenicity data for BBP no hepatocellular tumours were reported in mice and rats. However, an increased incidence of mononuclear cell leukemias was reported in female rats at high doses (12,000 ppm) of BBP, a marginally increased incidence of pancreatic adenomas and transitional epithelial papilloma of the urinary bladder was found in female rats. No increase in the incidence of tumours was reported in mice. Overall, BBP can be considered as a non-carcinogenic substance.

Regarding toxicity to reproduction, fertility as well as developmental studies are available. When taking the available data base into account a NOAEL at 100 mg/kg bw/day for effects on the reproductive organs/fertility from a 2-generation study in rats is used in the risk assessment (Nagao et al., 2000). The NOAEL is based on atrophy of the testis, epididymis, and seminal vesicle, and reduced reproductive organ weights at 10 or 18 weeks of age in the F1 generation at 500 mg/kg bw/day. In this two-generation study BBP was administered by gavage (0, 20, 100 and 500 mg/kg bw/day) to Sprague-Dawley rats. The results were as following; a significant reduction in fetal body weight was reported at 100 and 500 mg/kg bw/day on pnd 0. Furthermore, in male offspring (preweanling rats) a reduction in AGD (absolute), testis weight, epididymis weight, decreased FSH level and number of spermatogonia and spermatocytes in the seminiferous tubules was reported at 500 mg/kg bw/day. In postweanling rats at 500 mg/kg bw/day a decreased body, testis and epididymis weight was reported. Furhtermore, at 500 mg/kg bw/day, a delay in preputial separation in males, decreased testosterone and LH levels and increased incidence of testicular athrophy with decreased number of germ cells in the seminiferous tubules and decreased number of sperm in the epididymis was reported. In another recent 2-generation study (Tyl et al., 2004) significantly reduced mating and fertility indices were reported in F1 parents to make F2 offspring at 750 mg/kg bw/day. In the same study a significantly reduced relative and absolute paired ovaries and uterus weight was reported in F0 females. In adult F1 males a significant increase in reproductive tract malformations was reported (53.33% compared to 0% in controls). No increases in reproductive tract malformations were reported in females. Systemic toxicity reported at 750 mg/kg bw/day was limited to organ weight changes (liver, kidney) in males and females and histopathological lesions graded as minimal in females. The NOAEL for fertility was 250 mg/kg bw/day from this study.

For development a NOAEL at 50 mg/kg bw/day for offspring is used in the risk assessment (Tyl et al., 2004). This NOAEL value is based on a dose-related significant reduction in absolute and adjusted AGD in both F1 and F2 offspring from 250 mg/kg bw/day. At the next higher dose, 750 mg/kg bw/day a significant increase in F1 and F2 male pups with one or more nipples and/or areolae was reported. At weanling in F1 and F2 offspring a significant reduction in testis weight was reported. At post natal day 21 necropsies the percentage of males with reproductive tract malformations (RTM) were significantly increased in the F1 and F2 offsprings, and at adult necropsies the percentage of males with RTM were significantly increased in the F1 offspring (F2 offspring was not evaluated as adults). In F1 parental male a significant decrease in the testis, epididymis, prostate and seminal vesicle weight was reported (not evaluated in the F2 generation). The NOAEL for maternal toxicity was 750 mg/kg bw/day and was based on organ weight changes (liver and kidney) and histopathological lesions graded as minimal in the liver at 750 mg/kg bw/day. In this 2-generation study BBP was administered in the feed at doses of 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day. The developmental toxicity was also studied in mice exposed to BBP from gestation day (gd) 6 to 15 and sacrificed on gd 17. The NOAEL for offspring in this study was 182 mg/kg/day and are based on prenatal mortality and malformed foetuses at doses ≥ 910 mg/kg bw/day (NTP report, 1990). The maternal NOAEL value was 182 mg/kg/day. At the next higher dose level (910 mg/kg/day) a reduced dam weight gain (15%) with no reduction in adjusted body weight gain was reported. Due to the great

distance between the exposure groups in the mice study, the 2-generation study in rats is used in the risk assessment for developmental effects.

Only one human study is available where the relation between exposure to phthalates and semen quality was evaluated. In this study an association was found between high levels of mono butyl phthalate and/or mono benzyl phthalate in the urine and altered semen quality including semen concentration, semen motality and semen morphology (Duty et al., 2003). Due to the mixed exposure to various phthalates it is difficult to conclude that the effect observed on semen quality is related only to BBP exposure. Furthermore, the phthalates were only measured in a single spot urine sample in a relative small group of men (168) derived from subfertile couples. Due to the limitation of the study the NOAEL value for effects on reproductive organs in experimental animal studies will be used in the risk characterisation of BBP.

An association between prenatal and postnatal exposure to phthalates and whether the exposure had any influence on reproductive organ development in newborn boys was studied in two epidemiological studies. In the study by Swan et al. (2005) an association between maternal exposures to BBP as well as other phthalates and AGI in boys was reported. When comparing boys with prenatal MBeP (monobenzyl phthalate, reflecting exposure to BBP) exposure the odds ratio for a shorter AGI was 3.8. For the other monoester phthalates the odds ratio were 10.2 for MBuP (reflecting exposure to DBP), 4.7 for MEP (reflecting exposure to DEP), and 9.1 for MiBP (reflecting exposure to DINP) (all p-values < 0.05).

In the study by Main et al. (2005) no association was found between phthalate monoester levels (MEP, MMP, MBuP, MBeP, MINP and MEHP) in breast milk and cryptorchidism in newborn boys. However, a significant association was found between intake of milk contaminated with phthalates (MEP, MBuP, MMP and MINP) and postnatal surge of reproductive hormones (SHBG, LH, testosterone and inhibin B) in newborn boys. As regards the monoester metabolite of BBP, MBeP the tendencies were similar, however, they were not statistically significant.

These data support the hypothesis that prenatal phthalate exposure at environmental levels may affect male reproductive development in humans. However, due to the small sample size, (85 boys in Swan et al., 2005 and 130 boys in Main et al., 2005), further studies with larger sample size would have to be performed before clear conclusions can be drawn from these studies.

In conclusion, BBP is found to adversely affect the reproductive organs in experimental animal studies which may affect fertility. Furthermore, the substance is found to be a developmental toxicant and to possess anti-androgen like properties in experimental animal studies.

Endpoint	Study design	Critical effect	NOAEL/ LOAEL	Reference
Repeated dose toxicity	Wistar rats; 10/sex/group; 3 months; oral administration (in diet): 2500-12,000 (corresp. to approx. 151, 381, 960 mg/kg bw/day	Male rats: At doses ≥ 381 mg/kg bw/day kidney weight increase, gross morphological changes in the liver and histopathological changes in pancreas. At 960 mg/kg bw/day body weight decrease, slight anemia, liver weight increase and histopathologic changes in liver.	NOAEL: 151 mg/kg bw/day in male rats	Hammond et al. (1987)
Repeated dose toxicity	Sprague-Dawley rats; 25/sex/group; 13 weeks; inhalation: 51, 218 and 789 mg/m³	At 789 mg/m³ increase in relative liver and kidney weight in male and female rats. Decrease in serum glucose in male rats.	NOAEL: 218 mg/m³ in male and female rats	Monsanto (1982)
Reproduction toxicity, fertility/effects on the reproductive organs	Sprague-Dawley rats; two-generation study; 25/sex/group; administration by gavage; 0, 20, 100 and 500 mg/kg bw/day BBP	F_0: decrease i body weight gain in males at 500 mg/kg/day. A dose-dependent increase in kidney weight of both sexes, (significant from 100 mg/kg/day in females and at 500 mg/kg/day in males), a significant increase in liver weight in males at 500 mg/kg/day, and a significant decrease in ovary weight in females at 500 mg/kg/day. A decrease in testosterone (significant at 500 mg/kg/day), and increase in FSH (significant from 100 mg/kg/day) in males. F1: significantly decreased body weight at birth from 100 mg/kg/day, and at 500 mg/kg/day throughout the study. AGD was decreased and preputial separation delayed in males at 500 mg/kg/day. Macroscopic and microscopic changes of testis, and decreased testosterone levels at 500 mg/kg/day after puberty. Significantly decreased testis, epididymis, and seminal vesicle weight at 500 mg/kg/day in F1 postweaning. Decreased number of germ cells in the seminiferous tubules, and sperm in the epididymis at 500 mg/kg/day as well. BBP did not affect reproductive ability, including delivery and lactation.	No NOAEL value could be derived for effects on fertility. NOAEL for effects on the reproductive organs: 100 mg/kg bw/day	Nagao et al. (2000)

Endpoint	Study design	Critical effect	NOAEL/ LOAEL	Reference
Reproduction toxicity, development	CD Sprague-Dawley rats; 2-generation study; 30/sex/group; administration in feed; 0, 750, 3,750 and 11,250 ppm corresponding to approximately 0, 50, 250 and 750 mg/kg bw/day.	Development: reduced AGD from 250 mg/kg bw/day in F1 and F2 offspring. Weight changes in the reproductive organs in F1 and F2 male offspring, and macroscopic and microscopic lesions in the reproductive organs in male offspring at 750 mg/kg bw/day. Maternal toxicity: organ weight changes (liver and kidney), and histopathological lesions in the liver graded as minimal at 750 mg/kg bw/day.	NOAEL for developmental effects: 50 mg/kg bw/day based on reduced AGD from 250 mg/kg bw/day in F1 and F2 offspring. NOAEL for maternal toxicity: 250 mg/kg bw/day	Tyl et al. (2004)