European Union Risk Assessment Report

TRIS (2-CHLOROETHYL) PHOSPHATE, TCEP

CAS-No.: 115-96-8

EINECS-No.: 204-118-5

RISK ASSESSMENT

July 2009

FINAL APPROVED VERSION

Information on the rapporteur

Contact point:

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

Anmeldestelle Chemikaliengesetz

Friedrich-Henkel-Weg 1-25

44149 Dortmund

e-mail: chemg@baua.bund.de

This document is the last version of the Comprehensive Risk Assessment Report **Tris(2-chloroethyl) phosphate (TCEP)** a substance chosen from the EU 2nd Priority List.

Cataloguing data can be found at the end of this publication

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Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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

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

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

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0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 115-96-8

EINECS No. 204-118-5

IUPAC Name Phosphoric acid tris-(2-chlorethyl) ester

Synonym Tris (2-chlorethyl) phosphate, TCEP

Overall results of the risk assessment:

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

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

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

Summary of conclusions:

Environment

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

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

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

ad ii)

This conclusion applies to all life cycle steps to all environmental compartments, to the function of waste water treatment plants and to secondary poisoning via the food chain.

TCEP does not meet the PBT criteria.

A potential risk in future cannot be excluded if production and/or use volumes were to increase as a consequence of actions on other flame retardants. Therefore, it is recommended to monitor that the downtrend in use of TCEP is not reversed in future.

Human Health

Consumer

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

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

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

Ad iii) Risk reduction measures are required for babies with respect to the scenario sucking on toys taking into consideration the carcinogenic properties of the substance and the effects after repeated oral administration.

Workers

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

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

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

ad iii)

For TCEP three occupational exposure scenarios are evaluated. TCEP is produced (scenario 1) and is used for the production of formulations (scenario 2). The use of TCEP-containing formulations (scenario 3) includes spray application (scenario 3a) and applications without formation of aerosols (scenario 3b). The overall result of risk assessment indicates that

current exposure levels (inhalation and dermal contact) are too high for all occupational exposure scenarios.

From the toxicological point of view, concern mainly derives from the carcinogenic properties of TCEP. In addition, chronic toxicity and partly fertility impairment gives reason for concern.

Measures selected for risk reduction should be able to substantially reduce TCEP exposure of workers. Special emphasis should be given to the "spray application" scenario (dermal contact and inhalation).

With respect to risk assessment for carcinogenicity inhalation exposure at the workplace should be reduced to a level of 0.2 mg/m^3 or below. It is recommended to establish an occupational exposure limit for TCEP.

Concerning skin contact, exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Human exposed via the environment

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

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

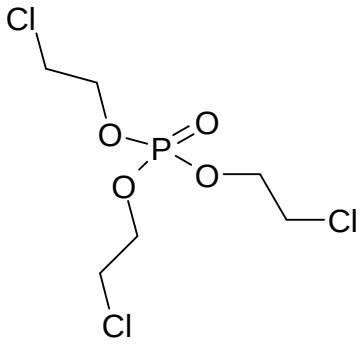
( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

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

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No.:	115-96-8
EINECS No.:	204-118-5
IUPAC Name:	Tris(2-chloroethyl) phosphate
Synonyms	Tris(2-chlorethyl)phosphat Ethanol, 2-chloro-, phosphate (3:1) Tris(β-chloroethyl) phospahte Tris(chloroethyl) phosphate Tris(2-chloroethyl) orthophosphate Tri(2-chloroethyl) phosphate Tri(β-chloroethyl) phosphate Tris(.beta.-2-chloroethyl) phosphate
Molecular weight:	285.49 g/mol
Empirical formula:	$C_6H_{12}Cl_3O_4P$
Structural formula:	



1.2 PURITY/IMPURITIES, ADDITIVES

Purity: 99.5 %

Impurities: water

1.3 PHYSICO-CHEMICAL PROPERTIES

Physical state	liquid at 20 °C	
Melting point	< -70 °C	Akzo Nobel (10.05.2000)
Boiling point	decomposition at 320 °C at 1013 hPa	Akzo Nobel (06.07.2000)
Density	1.4193 g/cm^3 at 25 °C	Akzo Nobel (15.06.2000)
Vapour pressure	43 Pa at 136.9 °C 0.00114 Pa at 20 °C (extrapolated)	Akzo Nobel (06.07.2000)
Surface tension	not determined	
Water solubility	7820 mg/l at 20 °C	Hazelton Europe (18.04.1994)
Partition coefficient	logPow = 1.78	Hazleton Europe (20.04.1994)
Flash point	200 °C at 1013 hPa	Courtaulds Chemicals (1996)
Auto flammability	480 °C	Hoechst AG (1994)
Flammability	not extremely flammable not highly flammable not flammable	CHEMSAFE
Explosive properties	not explosive (structural reasons)	
Oxidizing properties	not oxidizing (structural reasons)	

Vapour Pressure

The vapour pressure of a substance is defined as the saturation pressure above a solid or liquid substance. At the thermodynamic equilibrium, the vapour pressure of a pure substance is a function of the temperature only.

A vapour pressure of < 100 Pa at 20 °C was stated in the safety data sheets of the companies Bayer AG and Hoechst AG whereas Akzo Nobel give a value of < 40 Pa at 20 °C.

The value of 13,3 Pa at 25 °C was calculated by the Hoechst AG using the program "EPIWIN" version 2b of the Syracuse Research Corporation.

A value of $1.14 * 10^{-3}$ Pa at 20 °C was used for the risk assessment. This value is derived from the experimental determination of the vapour pressure at higher temperatures using the dynamic method. The measured values are in detail as follows:

0.43 hPa at 136.9 °C

0.99 hPa at 143.5 °C

2.03 hPa at 158.6 °C

5.00 hPa at 174.1 °C

15.03 hPa at 196.2 °C

The Clausius-Clapeyron equation was applied to these values for calculating the vapour pressure at 20 °C

Water Solubility

The water solubility of a substance is specified by its saturation concentration in pure water at a certain temperature, preferably at 20 °C.

The water solubility varies from 5 g/l to 7.8 g/l at 20 °C to in the safety data sheets of the companies Bayer AG, Hoechst AG, Akzo Nobel and Courtaulds Chemicals without giving further information on the test method or the testing procedure.

Moreover, the water solubility at 20 °C was determined experimentally using the flask method and result in a value of 7820 mg/l; the measured pH-values were in the range of 4.7 to 6.1. The test report was assessed as valid and plausible. Therefore the value of 7820 mg/l at 20 °C was used for the risk assessment.

Partition Coefficient

The partition coefficient (Pow) is defined as the ratio of the equilibrium concentrations (c) of a dissolved substance in a two phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$\text{Pow} = \text{equilibrium } c_{\text{n-octanol}} : \text{equilibrium } c_{\text{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (logPow).

Hoechst AG gives a logPow-value of 0.544 in their safety data sheet without any further information. Beyond that, Hoechst AG calculated the logPow-value of 1.47 using the program "EPWIN" version 2.0b of Syracuse Research Corporation. The calculated value of the company Akzo Nobel was 1.49 without information on the applied method or program.

For the risk assessment the value of 1.78 was applied. This logPow was determined experimentally by Courtaulds Chemicals in a GLP-test using the flask-shaking-method.

1.4 CLASSIFICATION

1.4.1 Current classification (Annex I of Directive 67/548/EEC)

Xn	Harmful
	Carc. Cat. 3
N	Dangerous for the environment
R40	Limited evidence of a carcinogenic effect
R22	Harmful if swallowed
R 51	Toxic to aquatic organisms
R 53	May cause long-term adverse effect in the aquatic environment

1.4.2 Proposed classification

In Germany TCEP is classified according to water-hazard class 2 (hazardous to water).

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- (Working group (Human Health) on classification and labelling of dangerous substances under Directive 67/548/EEC)

At the meeting November 2005 the EU classification and labelling working group (Human Health) agreed finally upon the following classification for TCEP:

Class of danger:	T	**Toxic**
	Carcinogen	Cat. 3
	Reproductive toxicant	Cat. 2
	N	Dangerous for the environment
	R 22	Harmful if swallowed
	R 40	Limited evidence of a carcinogenic effect
	R 51	Toxic to aquatic organism
	R 53	May cause long term effect in the aquatic environment
	R 60	May impair fertility

Currently tris(2-chloroethyl)phosphate is listed in Annex I to Commission for the 25th time Council Directive 67/548/EEC and has to be classified as Carcinogen Category 3 and labelled with Xn, (Harmful) and R 40 (Limited evidence of a carcinogenic effect).

The agreed classification proposal will be included in the next draft ATP.

2 GENERAL INFORMATION ON EXPOSURE

The environmental part of this risk assessment was first drafted in 1998. At that time TCEP was produced in the EU in quantities of about 2000 t/a. However, the situation has changed recently. Up-to-date information given by industry revealed that there is no production in Europe[4] anymore and processing has been reduced, but marketing of TCEP-containing products is still relevant for the EU.

The risk assessment is performed using import data from 2002. All life cycles are calculated using generic scenarios since no formulator and processor is known to the rapporteur.

2.1 PRODUCTION

There is no production in Europe at present (2001/2002). According to IPCS (1998) all commercial TCEP is produced by the reaction of phosphorus oxychloride with ethylene oxide followed by subsequent purification.

Past trends

According to the maximum range of production/import for 1991/1992, given in the IUCLID-database, an amount of 10 500 t/a was relevant at that time for the European market. TCEP production and use has been in decline since the 1980s as its historic use in rigid and flexible polyurethane foams and systems has been substituted by other flame retardants. According to IPCS (1998) global consumption of TCEP peaked at over 9000 t in 1989 but had declined to below 4000 t by 1997. In 1998 the EU tonnage was 2040 t of which 1950 t were produced in the EU, 580 t imported and 490 t exported.

Present situation

Specific information given by industry revealed that there is no production of TCEP in the EU at present, i.e. in 2001/2002. There are 3 companies importing a total of 1150 t TCEP in the EU (partly from Russia and Poland[5]). All of these importers are exclusively traders of TCEP. No specific information on formulation or processing could be obtained. However, the importing companies provided information on fields of application of their sales. These data are used in the calculation of the environmental exposure.

A tonnage of 143 t was exported outside the EU in 2002. The total EU tonnage present can be estimated to be **1007 t/a**. This quantity is used further in the risk assessment.

[4] Referring to countries of the European Union before enlargement at 1st May 2004.

[5] In the context of this Risk Assessment, Russia and Poland were considered as being outside the EU.

The rapporteur received information from a new EU member state[6] in 2005. This information has been voluntarily provided. It is used as additional information only. Production is estimated as 300 to 500 t for 2004. It is further stated that export to outside the EU is about 300 to 400 t for the same time period. The EU tonnage estimated above as 1007 t/a is used in the risk assessment.

TCEP is also formed as a reaction by-product in the manufacture of other commercial flame retardants in which TCEP has been declared an impurity. These flame retardants are currently undergoing ESR risk assessment (4th priority list, Rapporteur: UK/IRL). This additional amount of TCEP is considered only in the calculation of the regional background concentration (see Chapter 3.1.6).

2.2 PROCESSING / APPLICATION (CATEGORIES OF USE, AMOUNTS)

TCEP is used primarily as an additive plasticiser and viscosity regulator with flame-retarding properties for polyurethane, polyesters, polyvinyl chloride and other polymers.

Past trends

Historically the largest field of application of TCEP (80-90 % of the quantity produced) was that concerned with reducing the brittleness, and with the simultaneous flame-resistant finishing, of polyurethane in the production of celled, rigid or semi-rigid foam. The addition of 10 % TCEP relative to the finished foam is sufficient to achieve a clear flame retardant effect (GDCh, 1987).

On a small scale TCEP was also used as an intermediate for the production of wax additives (GDCh, 1987).

In GDCh (1987), further application fields of TCEP (10 – 20 % of total quantity) are given as follows[7]:

- Acetyl cellulose (10 - 70 % TCEP)
  - paints and varnishes
  - thermoplastics (foils, extrusion)
- Ethyl cellulose (foils)
- Nitrocellulose (paints and varnishes)
- Polyvinyl acetate (paints and varnishes)
- ,Polystyrole (adhesives for polyurethane foam)

[6] Acession to EU in May 2004.

[7] This information is only given for illustrative purposes.

- Polyvinyl chloride (20% TCEP at max.)

Present situation

There are indications that the market and respective application fields have changed over the past 15 years.

Currently TCEP is mainly used in the production of unsaturated polyester resins (~ 80 %). Other fields of application are acrylic resins, adhesives and coatings.

The main industrial branches to use TCEP as a flame-retardant plasticiser are the furniture, the textile and the building industry (roof insulation); it is also used in the manufacture of cars, railways and aircrafts.

Other utilisation of TCEP is represented by flame resistant paints and varnishes, e.g. for polyvinyl acetate or acetyl cellulose and the use as a secondary plasticiser for polyvinyl chloride to suppress the flammability resulting from plasticisers such as phthalates.

One company supplied quantitative information about the type of product and the application areas of their sales in 2002 (Table 2.1). However, these data are only representative for ~ 44 % of the total tonnage.

Table 2.1 Fields of application given by one company (representing 44 % of EU tonnage)

Type	Application are	%
Unsaturated polyester resin	Building industry, e.g. roofing	83
Acrylic resin	Roadside safety barriers	2
Adhesives	Building industry	5
Paints (wood and roofings)	Building industry, e.g. fire protection of wood	< 1
Polyurethane foam	Furniture	< 1
Others (unknown)	Unknown	9
Cellulose acetate	Transport	1
Textile coating	Upholstery	0

One importer indicated unsaturated polyester resins (75 - 80 %) and flexible foam (20 – 25 %) as application fields of their sales whereas another company stated no TCEP was used in any foam anymore. More detailed information was not available.

The fraction of TCEP used in paints and varnishes is unclear. Industry stated that there has been a move by the paint industry away from that use since TCEP was classified as toxic several years ago. A survey by the European Council of Paints, Printing Inks and Artists' Colour Industry (CEPE, 2002) amongst its members showed that 3 out of 10 companies responding still use TCEP in paints in Europe, without specifying any quantities. An update of this information by CEPE (2004) states that the total volume of TCEP in paints in the EU amounted to 10 t in 2003. The representativeness of that statement could not be verified. It is further stated that no new company has started the use of TCEP in paints after 2002, leaving three companies continuing the use of TCEP in paint manufacturing in 2003/2004.

The above Table 2.1 lists a quantity of < 1 % in paints. There is no use of TCEP in paints and varnishes listed in the SPIN data base (see below).

The information given by a new EU member state specifies use of TCEP in polyurethane foams and unsaturated polyester resins. Uses of TCEP in paints are not known.

The lead company commented on the unresolved issue of use of TCEP in consumer paints (Akzo Nobel, 2004). It is said that there is no need to put TCEP into consumer paints since there is no regulation governing the flammability of domestic paints. Keeping in mind the higher costs of TCEP compared to other plasticisers like phthalates TCEP would not be used as plasticisers either in consumer paints. In contrast, professional paints need for certain uses flame retardant properties, however theses are specialised products. The largest coating manufacturer in the world confirmed that no flame retardants are formulated into domestic paints.

Due to these somehow contradicting statements it is difficult to determine a quantitative breakdown of usage reflecting the present situation in Europe.

Specific information is given for 44 % of the total tonnage specifying that 1 % of that tonnage goes into paints. 5 % is used for intermediates, 94 % in the polymer industry. Industry tried unsuccessfully to get specific use information on the remaining quantity (56 %).

It can be assumed that no TCEP is formulated into consumer paints. Furthermore, no TCEP use in paints is given either in the SPIN database or in the data provided by the new member state. CEPE stated a total use of about 10 t/a of TCEP in EU for industrial paints. This corresponds to 1 % of the total EU tonnage in accordance with the specific information given for the 44 % of the EU tonnage. Summarising all this information the Rapporteur proposes to carry over the information on 44 % of the EU tonnage to the total amount arriving at the following scenarios.

Table 2.2 Tonnages in various scenarios

	total tonnage in application
paints	1 % (10 t/a)

polymers	94 % (947 t/a)
intermediate	5 % (50 t/a)

The resulting total mass balance and the respective industrial and use categories are shown in Table 2.3.

Table 2.3 Main, industrial and use category of TCEP

Main category (MC)	**Industrial category (IC)**	**Use category (UC)**	**Mass balance [in % of use]**
Use resulting in inclusion into or onto a matrix (II)	Polymers industry (11)	Flame retardants and fire preventing agents (22)	94
Use resulting in inclusion into or onto a matrix (II)	Paints and varnishes industry (14)	Flame retardants and fire preventing agents (22)	1
Non dispersive use (I)	Chemical industry (3)	Intermediate (33)	5

Product Register Data

The SPIN (Substances in Preparations In the Nordic countries) database was searched for information on TCEP in products on the national markets. The following data were found (Table 2.4):

Table 2.4 TCEP in products according to SPIN for 2001and 2003

country	2001		2003	
	number of preparations	tonnage	number of preparations	tonnage
Norway	13	1104	8	1285
Sweden	11	9	12	9
Finland	14	306	7	0.2
Denmark	25	190	13	4

The above tonnages seem unrealistically high compared to the total identified EU tonnage. The reason is the way the data are recorded in SPIN:

The total amount of a substance in SPIN is the added quantity of the substance in all products the export amount subtracted. That is to say that if a substance is registered first as the imported raw material and then as part of the final preparation the quantity will be counted twice. Substances that are used for formulation of chemical products and that are imported, and most are in the Nordic countries, will thus be accounted for with maybe double the actual amount.

Another factor giving a distortion of the quantity value is when concentration has been registered as an interval. In such cases the upper limit has been chosen for calculations of the substance amount in Denmark, Finland and Norway. Depending on how wide the allowed interval is in the different countries the discrepancy between the given value and the true value will vary. For example the tonnage interval given for Norway in 2001 ranges from 61 t (min) to 1104 t (max).

Therefore, the tonnages in Table 2.4 have to be considered overestimations.

The tonnages notified in Denmark and Finland have gone down considerably between 2001 and 2003. Norway showing a notably high tonnage compared to the other countries in 2001 registered an even higher tonnage in 2003. Reasons may be due to the data recording explained above. However, it has to be noted that TCEP was notified as being present in consumer products in 2001 and 2002 but not in 2003.

Main industrial use categories (2001) are given as "Manufacture of rubber and plastic products" (Norway, 538 t, 5 preparations; Finland, 403 t, 5 preparations) and "Manufacture of chemicals and chemical products" (Norway, 41 t, 4 preparations). Specifying the technical use of these preparations, "Adhesives, binding agents" (Norway, 5 preparations) and "Flame retardants and extinguishing agents" (Denmark, 4 preparations) are identified as the main fields of application.

3 ENVIRONMENT

3.1 Environmental exposure

3.1.1 Release into the environment

On the local scale releases of TCEP are expected during the industrial use of the polymer components as well as the formulation and industrial use (processing) of paints and varnishes. The less relevant use of TCEP as an intermediate should be considered additionally. Although there is no production in Europe a generic scenario for production is performed.

The flame retardant TCEP is physically combined with the polymer matrix. Therefore, TCEP could migrate to the surface. Releases might be expected during service life and disposal of products containing TCEP.

There is a recently published emission scenario document (ESD) on plastic additives: "Emission Scenario Document on Plastics Additives" (OECD, 2004). The document gives loss factors for flame retardants contained in plastic materials. However, these relate to solid flame retardants whereas TCEP is in the liquid state at room temperature. Although not appropriate for assessing losses for raw materials' handling and compounding, the ESD is used to estimate diffuse releases originating from service life and disposal. The ESD is also used to estimate diffuse losses from production and use of V6 where TCEP is present as impurity. These diffuse emissions are accounted for in the calculation of the regional background concentration. It has to be kept in mind that - in absence of further information - many of the assumptions made are "best guesses".

There is also an ESD for IC 14 Paints, Lacquers and Varnishes Industry available in Chapter 7 of the TGD (EC, 2003a).

A more elaborate coating emission scenario document "Chemicals used in the Coatings Industry: Paints, Lacquers and Varnishes" is currently being developed (EA, 2003). Difficulties in the application arise due to very limited information regarding the use of TCEP in the coating sector. Nevertheless, it is used to estimate the loss of TCEP to water and air during the formulation of paints (see 3.1.3).

ESDs provide very valuable information on branch and process specific emissions. However, difficulties in the application for this risk assessment arise due to limited information regarding the exact use of TCEP. If as a result too many (unconfirmed) assumptions had to be made, the ESD would not be any more realistic than generic calculations.

To nevertheless make the best use of the ESDs available, the Rapporteur decided on the following:

- ESD for Coating Industry (EA, 2003) is used to estimate the loss of TCEP to water and air during the formulation of paints, and compared with generic calculation using A- and B-Tables from the TGD (EC, 2003a) (see 3.1.3).

- ESD on Plastics Additives (OECD, 2004) is used to estimate emissions of TCEP during service life and disposal of polymers containing TCEP as well as diffuse releases of TCEP stemming from production, formulation (e.g. materials handling and

compounding) and processing (e.g. conversion and moulding) including service life of V6. The estimated emissions are used for the calculation of the regional background concentration.

3.1.2 Environmental fate

Degradation

Biodegradation

Only a limited number of test results on aerobic and anaerobic biodegradability of TCEP are available.

In a modified Sturm test (OECD 301 B) on ready biodegradability with 10 mg/l and 20 mg/l TCEP a DOC removal of 13 % and 4 %, respectively, could be achieved after 28 days (Akzo, 1990a). However, cumulative CO_2 production by day 28 at either concentration was negligible with a maximum of 1 % of $ThCO_2$ at 20 mg/l.

In a MITI-I test (OECD 301C) employing sludge from different sewage treatment plants, rivers, bays and a lake as inoculum a biodegradation of 4 % after 28 days was obtained. Biodegradation was measured as BOD (CITI 1992). In a Zahn-Wellens test (OECD 302 B) with industrial activated sludge the elimination amounted to 15 % after 21 days. 11 % elimination after 1 h could be accounted to adsorption (Hoechst, 1978). In a second Zahn-Wellens test with industrial activated sludge the elimination after 27 days was < 10 % (Hoechst, 1985).

The anaerobic biodegradation of TCEP was tested according to ISO DIS 11734 with a concentration of 80 mg/l related to DOC. No degradation could be observed after 58 days (Noack, 1993).

The primary degradation of TCEP in a concentration of 5 mg/kg soil was investigated in a laboratory test system with standard soil for 100 days. The degradation kinetic curve could be fitted to a 2^{nd} order square root function resulting in a DT_{50} and DT_{90} of 167 days and >>100 days, respectively. (Römbke et al., 1995)

Concluding from these results, TCEP must be considered as non biodegradable.

Although a certain low elimination may be expected under environmental conditions, the rate constant applied for the assessment of the elimination by biodegradation in municipal sewage treatment plants, surface waters, soils and sediments has to be set to zero as a realistic worst case (Table 3.1).

Table 3.1 Degradation constants of TCEP in different compartments

Compartment	degradation constant
Waste water treatment plant	$kbio_{WWTP} = 0\ h^{-1}$

Aquatic environment	$kbio_{SW}$ = 0 d^{-1}
Sediment	$kbio_{SED}$ = 0 d^{-1}
Soil	$kbio_{SOIL}$ = 0 d^{-1}

Hydrolysis and Photolysis

Due to investigations by Brown et al. (1975) TCEP has a hydrolysis half-life of 3980 days (≈ 11 a) at pH = 7. Two further data at pH 3 (no hydrolysis) and at pH 10 ($t_{1/2}$ = 101 d) are given in the same reference. Therefore it can be concluded, that neither for the local nor for the regional scale hydrolysis contributes to the environmental degradation of TCEP.

A direct photolysis in water is not expected due to the molecular structure of TCEP, i.e. there is no relevant absorption above a wavelength of 290 nm.

Photooxidation in air

An estimation of the half-life for the atmospheric reaction of TCEP with hydroxyl radicals with the program AOP 1.65 yields a value of 17.5 h (24-h day, $5 \bullet 10^5$ OH/cm^3). Experimental studies are not available. With this estimated half-life it is expected that the photooxidation in air represents a major degradation path for TCEP in the environment.

Distribution

With a Henry's law constant of $4.155 \bullet 10^{-5}$ Pa • m^3 • mol^{-1}, TCEP has a low volatility.

Since no experimental results on the adsorption of TCEP to soil are available, the estimation of the adsorption coefficients to soil, sediment, suspended matter and sewage sludge are performed due to the TGD, using a log K_{ow} of 1.78 and calculating a K_{OC} of 110.2 l/kg with the appropriate SAR-equation (see Appendix 1).

The TGD does not differentiate between various types of phosphates. Basis is the K_{OW} since hydrophobic interactions are the most prominent type between non-polar chemicals and soil. However, more specific interactions of adsorption mechanisms are possible which might be better reflected in measurements.

Within the ESR assessment of other chloroalkyl phosphates (4^{th} priority list; Rapp: UK) K_{OC} values were measured by HPLC according to OECD guideline 121. Results are listed in Table 3.2 and compared with K_{OC} values calculated according to TGD on the basis of log K_{OW} using QSAR for phosphates. In addition, there is a measured K_{OC} value for TDCP according to OECD guideline 106. An empirical relationship for dependence of log K_{OC} from log K_{OW} was derived and used to read across from TDCP to TCPP and V6. The resulting K_{OC} values are also listed in Table 3.2. (EC, 2006a).

Table 3.2 Comparison between measured and calculated K_{OC} of some chloroalkyl phosphates

Substance (CAS)	K_{OC} derived from OECD 106 result for TDCP [l/kg]	K_{OC} measured by HPLC [l/kg]	K_{OC} estimated acc. to TGD (phosphates) [l/kg]
TCPP (13674-84-5)	174	576	304
TDCP (13674-87-8)	1780	12300	951
V6 (38051-10-4)	245	11000	360

Source: EU Risk Assessment Reports on TCPP, TDCP and V6 (Drafts, 2006)

It is apparent that all values estimated from HPLC are higher than the predicted values from the TGD and values derived from OECD 106 for TDCP. Both the latter are within the same order of magnitude. The 7-fold higher K_{OC} (TDCP) from the HPLC test suggests that some specific interaction with the HPLC column had occurred. (EC, 2006a). It was agreed to use the K_{OC} values derived from OECD 106 for TDCP for the risk assessments of these chloroalkyl phosphates.

Applying the same empirical relationship of log K_{OC}=-0.44+logK_{OW} to read across to TCEP would result in a K_{OC} of 22 l/kg. This value is smaller than the one derived from TGD. However, uncertainty in reading across from TDCP to TCEP might be similar or higher than uncertainty in applying QSAR methods.

The estimated K_{OC} of 110.2 l/kg is used further in the risk assessment.

According to the classification scheme by Blume and Ahlsdorf (1993) the adsorption of TCEP is classified as being ‘low’.

The partition coefficients are summarised in the following table:

Table 3.3 Partition coefficients of TCEP

Compartment	**Partition coefficient**
soil (2% OC)	K_{p_soil} = 2.2 l/kg
sediment (5 % OC)	K_{p_sed} = 5.5 l/kg
suspended matter (10 % OC)	K_{p_susp} = 11.0 l/kg
activated sludge (37 % OC)	K_{p_sludge} = 40.8 l/kg
raw sewage (30 % OC)	K_{p_sewage} = 33.1 l/kg

Using the multimedia fugacity model EQC (level 1, type 1) for the equilibrium distribution of TCEP between the different environmental compartments, the following results are obtained:

Table 3.4 Distribution of TCEP according to EQC model

Compartment	Percentage
Air	<0.001
Water	94.8
Soil	5.06
Sediment	0.11

Based on the physiochemical properties of TCEP, the water compartment is the main target of distribution. Distribution into soil/sediment is much lower; partitioning into air can be neglected.

Bioaccumulation

The log K_{OW} of 1.78 indicates a low bioaccumulation potential. This is confirmed by experimentally determined bioaccumulation factors.

In a bioaccumulation study with *Cyprinus carpio* a BCF of 0.6 - 0.8 and < 1.2 - 5.1 was found after 42 days exposure to 1 mg/l and 0.1 mg/l, respectively (CITI, 1992).

In a static test with goldfish (*Carassius auratus*) at 0.9 mg/l (1 % of LC50) a BCF of 0.9 was achieved after 96 h. In the same test system at 2.1 mg/l with killifish (*Oryzias latipes*) a BCF of 2.2 was attained (Sasaki et al., 1981).

In a flow-through system with killifish at 2.1 - 2.4 mg/l uptake was rapid and maximum concentration was reached after 1 day. During 11 days concentration in fish remained constant resulting in BCFs of 1.2 - 1.4. At 12.7 mg/l during 5 days a BCF of 1.1 was recorded resulting in a tissue concentration of 14 mg/kg. Depuration was rapid with a half-life time of 0.7 h (Sasaki et al., 1982).

Elimination in waste water treatment plants

Based on the cited physical chemical properties in Chapter 1, as well as the biodegradation rate of 0 h^{-1} in the WWTP, the elimination through biodegradation, adsorption and volatilisation can be estimated with the model SimpleTreat 3.0. Due to this calculation model 98.6 % of TCEP remain in the water and 1.4 % is adsorbed to sludge. There is no elimination of TCEP by volatilisation into the atmosphere (Appendix 1).

Table 3.5 Elimination in WWTP

Evaporation to air (%)	0
Release (dissolved) to water (%)	98.6
Adsorption to sewage sludge (%)	1.4
Degradation (%)	0
Total elimination from water (%)	**1.4**

3.1.3 Aquatic compartment

(incl. sediment)

3.1.3.1 Estimation of $Clocal_{water}$ / Generic approach for production and intermediate processing

For the industrial production of TCEP, ethylene oxide is added to phosphorous oxychloride in the presence of a catalyst. For purification and removal of the catalyst the crude TCEP is washed with aqueous-acid or aqueous-alkaline solutions and subsequently with water (GDCh, 1987).

The Technical Guidance Document (EC, 2002) proposes a generic (i.e. non site-specific) calculation for the release of chemicals to surface waters. Using the import/export data of 2002, 1007 t/a TCEP are relevant for the EU.

In a first approach it is assumed that 1 000 t/a are covered by one producer. An amount of 5 % of the regional tonnage of 503 t/a (see Chapter 2.2, Table 2.3 and Chapter 3.1.1.2, Figure 3.1) is assumed to be processed as intermediate at the same site. This quantity corresponds to 25 t/a.

A $Clocal_{water}$ of 28.76 µg/l is calculated for the generic production and processing of TCEP (Input: T_{prod} = 1000 t/a; f_{prod} = 0.3 %; T_{proc} = 25 t/a; f_{proc} = 2 %; $EFFLUENT_{STP}$=10000 m^3/d; DILUTION=40; Temission = 300 d/a; elimination 1.4 %).

Although standard procedure this approach has to be considered worst case since there is no production of TCEP in the EU anymore. For comparative purpose, a $Clocal_{water}$ of 4.11 µg/l was calculated for the processing only using the same input data as above.

3.1.3.2 Estimation of $Clocal_{water}$ / Generic approach: use

To calculate the generic, local concentrations in surface water resulting from the use of TCEP, the use pattern and percentages given in Chapter 2.2, Table 2.3 are used, i.e. 94 % polymers, 1 % paints and varnishes and 5 % intermediates.

With the available information on the TCEP content in the respective formulations and products, a content of about 10 wt % for polymers and paints is assumed for this initial estimation.

It is further assumed that half of the EU continental use volumes are attributable to a highly industrialized region. The reasoning behind this assumption is firstly the 'low' consumption amount of 1007 t/a. Secondly, the industrial use of TCEP can be considered fairly specialised with industry indicating further reduction in use of TCEP in future.

The following scheme (Figure 3.1) summarises the proposed scenario for calculating the generic local concentrations for the use of TCEP, mainly using the A/B-tables from the TGD. The amount of TCEP contained in different applications is given in brackets. The respective tonnage of paints (formulation volume: 50 t/a) and polymers (formulation volume: 4730 t/a) is used to obtain the fraction of main source and the duration of the emission from the B-tables.

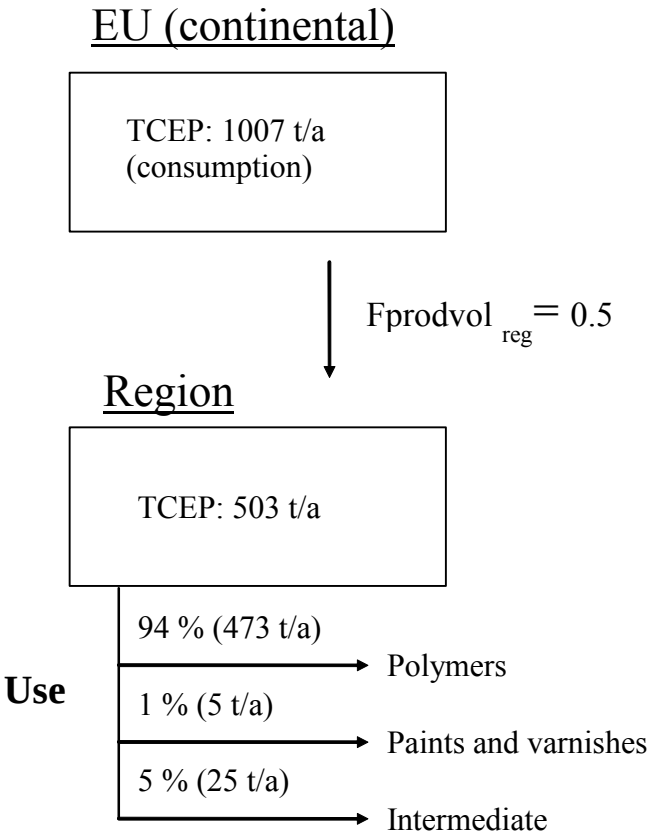

Figure 3.1 Schematic geographical representation of use of TCEP

An overview over the various use scenarios and the calculations of $Clocal_{water}$ is shown in Table 3.6 and a brief description is given subsequently.

Table 3.6 Data used for aquatic exposure assessment from the use of TCEP

Use of TCEP	**Polymers industry**	**Paints and varnishes**	**Paints and varnishes**
Tonnage (t/a), regional	473	5	5
Industrial category Use category	11 (Polymers industry) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)
Life cycle step	processing	formulation	industrial use (processing)
Release factor to water	0.0005 (A-table 3.11; I, A)	0.02 (A-table 2.1)	0.05 (A-table 3.15), water based
Fraction of main source	0.15 (B-table 3.9)	1 (B-table 2.10)	0.6 (B-table 3.13)
Number of days	284 (B-table 3.9)	300 (B-table 2.10)	200 (B-table 3.13)
Size of STP (m^3/d)	2 000	2 000	2 000
Dilution in receiving water	10	10	10
$Clocal_{effl.}$ (µg/l)	61.6	164	370
$Clocal_{water}$ (µg/l)	6.16	16.4	37.0

Processing of polymers

For the processing of polymers a generic calculation is performed using the A/B-tables for default emissions.

-- IC: 11 (Polymers industry); UC: 22 (Flame retardants), Category A, type I

The result of this estimation is $Clocal_{water}$ = 6.16 µg/l.

Formulation of paints and varnishes

For the formulation of paints and varnishes a generic calculation is performed using the A/B-tables for default emissions.

To derive the fraction of main source from the B tables, the tonnage is related to the formulated paints of 50 t/a.

-- IC: 14 (Paints and varnishes industry); UC: 22 (Flame retardants)

The result of this estimation is $Clocal_{water}$ = 16.4 µg/l.

There is an Emission Scenario Document (ESD) on "Chemicals used in the Coatings Industry: Paints, Lacquers and Varnishes" available (EA, 2003). In Chapter 3.1.3 this ESD is used to estimate the loss of TCEP to water and air during the formulation of paints.

Industrial use (processing) of paints and varnishes

For the industrial use of paints and varnishes a generic calculation is performed using the A/B-tables for default emissions. To derive the fraction of main source from the B tables, the tonnage is related to the formulated paints of 50 t/a.

-- IC: 14 (Paints and varnishes industry); UC: 22 (Flame retardants)

-- water based paints

The result of this estimation is $Clocal_{water}$ = 37.0 µg/l.

3.1.3.3 Sediment

The calculation of the PEC_{local} for sediment is done using the highest local aquatic exposure of $PEClocal_{water}$ = 37.0 µg/l (industrial use of paints):

PEC_local_{sed} = 0.118 mg/kg (wet weight) = 0.306 mg/kg (dry weight)

Measured sediment concentrations are reported under „Monitoring data“.

3.1.3.4 Monitoring data

Surface water

A synopsis of monitoring data for TCEP is given in GDCh (1987), concerning the river Rhine for German and Dutch locations. The reported values measured between 1972 and 1986 are generally in the range of 0.1 µg/l to 1 µg/l.

A detailed monitoring study for TCEP in the river Rhine and its tributaries was performed by Knepper and Karrenbrock (1996). With a detection limit of 0.01 µg/l, TCEP was found to be a permanent pollutant in surface waters. Concentrations at the location of Cologne were between 0.05 and 0.3 µg/l. Using the measured concentrations a load of 20 - 25 kg/d was calculated by the authors. For the tributaries of river Rhine, including the rivers Neckar, Main, Mosel, Lahn und Ruhr, concentrations ranging from 0.18 to 1.3 µg/l were determined, i.e. generally higher concentrations than had been measured in the Rhine.

A similar monitoring program was performed for the river Elbe and its tributaries (ARGE, 2000). TCEP was monitored in 1996 and 1998 at 7 sites along the river (6 measurements per year). Concentration of TCEP was found in ranges of 10 (min) – 220 (max) ng/l.

Spot tests on surface waters (i.e. 2 major rivers (Rhine and Neckar) as well as various smaller rivers and canals) above and below the discharge point of a municipal WWTP were performed in Baden-Wuerttemberg/Germany (Metzger and Möhle, 2001). TCEP was not at all detected at 5 out of 10 locations (detection limit: 0.1 µg/l); for a further 2 sites that was the case after the WWTP. Maximum concentration detected was 0.27 µg/l.

Fries and Püttmann (2003) monitored TCEP in the Oder river near the eastern border of Germany. At four different sampling times (March 2000, November 2000, March 2001, July 2001) nine river water samples were collected. Mean values ranged from 30 ng/l (March 2000) to 282 ng/l (March 2001). In July 2001 the measured concentrations were generally higher (554 – 1236 ng/l).

The same authors had monitored TCEP in various rivers in Germany (River Rhine at Rüsselsheim; R. Elbe at Hamburg and Dresden; R. Main at Frankfurt; R. Oder at Küstrin; R. Nidda at Frankfurt; R. Schwarzbach at Rüsselsheim) in an earlier study (Fries and Püttman, 2001). The concentrations ranged from non-detectable levels (< 1 ng/l) up to 220 ng/l (51 samples taken in March and November 2000). In comparison to concentrations of TCEP of 138 to 3000 ng/l measured previously in selected rivers (references cited therein from 1990-1999), the authors note a decreasing trend in environmental concentrations of TCEP.

Concentrations of TCEP of <12 to 80 ng/l were measured in the River Ruhr basin/Germany (Bester et al., 2003)

Measurements during 1999-2000 of 95 organic wastewater contaminants in 139 streams across 30 U.S. states were recently published (Kolpin et al., 2002). The sampling sites focussed primarily on areas considered susceptible to contamination from human, industrial and agricultural waste water and represent a wide range of geography, hydrogeology, land use and climate. TCEP was detected in about 60 % of the samples. The maximum concentration was 0.54 µg/l.

Prösch et al. (2002) examined 29 bath lakes in Mecklenburg-Western Pomerania/Germany, a rural area with mainly agricultural utilisation. No TCEP was detectable in 18 of these randomly chosen lakes (detection limit: 0.01 µg/l; one-time sampling). The maximum measured concentration of 0.09 µg/l is assumed to stem from a contaminated site.

In Italy, Galassi (1991) reported concentrations ranging from < 10 ng/l up to 293 ng/l at one station at River Po and two marine stations in the Adriatic Sea within 4 sampling campaigns carried out from April to August 1988.

Monitoring data for the Midland region near Derby/UK was supplied for the years of 1990 to 2003 (Environment Agency, 2003). Measurements were taken around two locations presumably discharging TCEP. At one of the sites a company used to produce a flame retardant. The other location received effluent of a textile finishing company. Both companies have closed down in recent years however TCEP can still be detected. Whereas in early 1990s maximum values of up to 4 mg/l were measured, TCEP concentration has been constantly < 5 µg/l since around 1998 (90 percentile of all values: 3.55 µg/l). Values from January 2004 to July 2005 from the same region showed a 90 percentile of 1.03 µg/l (Environment Agency, 2005).

Precipitation

Chloroorganics were analysed in samples of rain (Ireland), snow (Poland, Southern Sweden, Antarctica) and glacier ice (Northern Sweden) (Laniewski et al., 1998). The sites were selected to represent both urban and remote areas. Apart from the surficial snow sample from Antarctica, TCEP was present in all other samples in concentrations ranging from 1 – 21 ng/l.

A TCEP concentration of 121 ng/l was detected in one sample of rain water from the Oderbruch area/Germany (Fries and Püttmann, 2003).

WWTP effluent

TCEP was measured in the effluent of 3 communal WWTP in Saxony-Anhalt and Saxony (Germany) in 1999 and found in concentrations between < 50 and 600 ng/l (ARGE, 2000).

Prösch et al. (2000) studied the occurrence of TCEP in effluents of 13 municipal WWTP in the German Baltic Sea catchment area. TCEP was detectable in all WWTPs in concentrations of 0.06 µg/l (min) to 1.66 µg/l (max), based on one measurement per month. The effluent discharge rate varied between 137 and 44380 m^3/d.

Comparable results were found in a study in Baden-Wuerttemberg, another federal state of Germany (Metzger and Möhle, 2001). Values between < 0.1 µg/l (detection limit) and 1.88 µg/l (max) were detected in 22 WWTP in Spring/Summer 2002 (one-time sampling).

TCEP was measured in October 2002 (5 samples) and February/March 2003 (7 samples) in the influent and effluent of 2 communal WWTP in North Rhine Westphalia/Germany (MUNLV NRW, 2003). The measured values (median; influent/effluent) were 0.22/0.25 µg/l at Düsseldorf and 0.32/0.36 µg/l at Cologne, respectively. The authors could not explain the higher concentration of the effluent compared to the influent.

In July 2001, influent and effluent waste water samples (one-time sampling) were collected from three municipal WWTP and one industrial WWTP discharging their treated waste water into the Oder River (Fries and Püttmann, 2003). The mean concentrations of TCEP with regard to municipal WWTP were 986 ng/l (influent) and 352 ng/l (effluent), respectively. Interestingly, these concentrations are higher compared to the industrial WWTP (influent: 568 ng/l; effluent: < 1 ng/l (detection limit)) although representativeness is questionable.

A recent study determined the elimination efficiency with respect to various chlorinated and non-chlorinated organophosphorous flame retardants in two WWTP in the Ruhr/Rhine area in Germany (Meyer and Bester, 2004). Samples were taken in spring 2003 as 24-hour composite samples (automatic sampling; LOD: 6.1 ng TCEP/l. No elimination of TCEP was observed. Mean effluent concentrations were 350 ng/l and 370 ng/l, respectively.

Ground water

Ground water was investigated by Metzger and Möhle (2001) in Baden-Wuerttemberg/Germany (one-time sampling). One sample was taken at ca. 3 m depth at an uncontaminated sited and showed no TCEP (detection limit: 0.01 µg/l). At another site two samples were taken at 31 and 44 m below a landfill site. Originally a quarry this site was used as dumping ground between 1962 and 1977. About half of the waste originated from households (70 %) and industry (30 %). That was mixed with soil and construction waste. Concentrations of TCEP were found to be 0.37 µg/l (31 m depth) and 0.10 µg/l (44 m depth).

The same authors performed spot checks on landfill leachates originating from household landfills (3 sites) as well as from one hazardous waste site. The leachates stemming from household landfills showed TCEP concentrations of 0.17 and 0.19 µg/l. Two of the sites treat the leachates with activated charcoal before discharging them into municipal WWTPs. TCEP was not detectable in the effluent of the activated carbon treatment. TCEP concentration of the hazardous waste site leachate was 5.56 µg/l but again after treatment with activated charcoal no TCEP was detectable.

The Environment Agency (2005) of England and Wales analysed the concentration of TCEP in leachate from 22 landfills in southern England and Wales during Spring 2005. The data obtained are still being analysed. Nevertheless, these early data show that TCEP was only detected in one sample at 0.245 µg/l (detection limit of 10 µg/l for majority of measurements).

Fries and Püttmann (2003) collected 76 ground water samples at 3 different sampling times between March 2000 and March 2001 in the Oderbruch area located approximately 70 km

east of Berlin. Around 25 monitoring wells (1.5 to 19 m depth) situated around 2 main sampling sites showed mean values of < 1 ng/l (detection limit) to 312 ng/l.

For 12 wells, located in a distance of 600 m from the bank of river Rhine, concentrations of about 0.17 µg/l at maximum were measured (Knepper and Karrenbrock, 1996). In a recent study 573 house wells in rural areas of Mecklenburg-Western Pomerania/Germany were investigated (Prösch et al., 2002). TCEP was only detected at 2 wells (detection limit: 0.01 µg/l) with a maximum concentration of 0.18 µg/l.

During 2004-2005 the Environment Agency (2006) conducted a survey on groundwater samples in England as part of a speculative monitoring to identify any contaminants. TCEP was measured in 61 samples. Concentrations ranged from 0.02 to 1.43 µg/l (90 percentile of all values: 0.33 µg/l). However, these results must be treated with caution since this was a screening exercise only using GCMS.

Sewage sludge

Sewage sludge samples from 10 German treatment plants were investigated by Weisser in 1992. Concentrations of < 0.5 mg/kg up to 11 mg/kg (dry weight) were found with a mean value of 3.1 mg/kg.

Sediment

In a paper published by the Niedersächsisches Landesamt für Ökologie (1997) TCEP concentrations for ten sediments from five German rivers (Elbe, Jeetzel, Leine, Oker and Aller) in Lower Saxony are reported. The measured concentrations ranged from 5.4 to 15.0 µg/kg (dry weight) with a median value for the ten sites measured of 8.3 µg/kg (dry weight). From this median value a concentration in surface water of 1.0 µg/l can be calculated at equilibrium according to the method described in the TGD. Concentrations of 0.5 – 100 µg/kg (dry weight) were monitored in the sediment of the river Elbe (ARGE, 2000).

In 1999 sediment samples of the 3 great rivers Rhine, Danube and Neckar in Baden-Wuerttemberg (Germany) were analysed for TCEP (Metzger and Möhle, 2001). Sampling was concentrated on freshly deposited sediment, i.e. the top layer, and took place only once. TCEP was not detectable in 6 out of 12 sites allocated at different spots of the rivers (detection limit: 20 µg/kg dry weight). The maximum concentration found was 188 µg/kg (dry weight).

3.1.3.5 Comparison between predicted and measured levels

Most of the available monitoring data were measured in Germany, partly in areas of small size and often done as one-time sampling. Representativeness might be questionable.

Most of the concentrations measured recently in surface waters (0.01 – 0.3 µg/l) are of comparable magnitude to the modelled $PECreg_{water}$ (0.087 µg/l).

Measured sediment concentrations are scarce and a comparison with predicted concentrations cannot be made.

3.1.4 Atmosphere

3.1.4.1 Estimation of $Clocal_{air}$ / Generic approach: production and intermediate processing

No ESD for the release of chemicals into the atmosphere during production/processing is available at the moment. The emissions are therefore estimated with the emission tables presented in Appendix I of the Technical Guidance Document.

An emission factor of zero is given in Table A1.2 for all MC's for a vapour pressure < 1 Pa, i.e. default emission during production is zero.

The emission factor for the processing step is also zero (Table A3.3).

Therefore generic estimation results in zero atmospheric emissions during production/ processing.

3.1.4.2 Estimation of $Clocal_{air}$ / Generic approach: use

The following table summarizes the results of the calculations of $Clocal_{air}$ and $DEPtotal_{ann}$ for the use of TCEP performed according to the TGD.

Table 3.7 Data used for atmospheric exposure assessment

Use of TCEP	**Polymers industry**	**Paints and varnishes**	**Paints and varnishes**
Tonnage (t/a), regional	473	5	5
Industrial category Use category	11 (Polymers industry) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)
Life cycle step	processing	formulation	industrial use (processing)
Release factor to air	0.0005 (A-table 3.11, I, A)	0.001 (A-table 2.1; 1c)	0 (A-table 3.15), water based
Fraction of main source	0.15 (B-table 3.9)	1 (B-table 2.10)	--
Number of days	284 (B-table 3.9)	300 (B-table 2.10)	--
$Clocal_{air}$ (µg/m³)	0.035	0.005	0
$DEPtotal_{ann}$ (mg m^{-2} d^{-1})	1.23×10^{-4}	1.74×10^{-5}	0

3.1.4.3 Monitoring data

No monitoring data are available for the air compartment. Aston et al. (1996) detected TCEP in pine needle samples collected in the Sierra Nevada foothills/California (USA). Concentrations were up to 3 times higher (10-1950 ng/g_{wwt}) compared to those reported for aqueous matrices in other studies Aston et al. refer to.

The authors discuss their findings and suspect that aerial transport and deposition from nearby point sources may play a role. They argue that although organophosphate pesticides are used on California's orchards and volatilisation of these compounds has been demonstrated, the source of haloalkyl organophosphate vapours is unknown. They further discuss that it is possible that landfills or incineration sites of waste plastic articles emit TCEP into the air. Uptake into the plant might also occur via vapours volatilising from soil after being deposited there. Due to a general lack of data in that field it is not possible to broaden this discussion at this point.

3.1.5 Use of Emission Scenario Document for loss estimation to air and water (formulation of paints)

As mentioned in Chapter 3, there is an Emission Scenario Document (ESD) on "chemicals used in the coatings industry: paints, lacquers and varnishes" available (EA, 2003). Difficulties in the application arise due to limited information regarding the use of TCEP in the coating sector. Nevertheless, the ESD is used to estimate the loss of TCEP to water and air during the formulation of paints.

Since information is lacking regarding the exact conditions at formulation, the following assumptions are made:

Table 3.8 Assumptions made for application of ESD

condition	**value (unit)**
type of paint	water-based
tonnage TCEP used per site	5 t/a
tonnage paint used per site	50 t/a
number of batches	1 batch/day
number of days	300 days/a
loss to water per batch	0.5 %
loss to air per batch	0.164 %*

* for middle air speed, high boiling point substance, no lid fitted to vessel

Using the values above and standard WWTP with 1.4 % removal and standard dilution by 10, a $Clocal_{water}$ of 4.11 µg/l is calculated. For air, the $Clocal_{air}$ is 7.6 x 10^{-3} µg/m³.

These values compare very well with $Clocal_{water}$ of 16.4 µg/l and $Clocal_{air}$ of 5 x 10^{-3} µg/m³ derived for the formulation of paints using the A- and B-tables of the TGD (EC, 2003a), thereby backing these.

The application of the ESD is based on a number of assumptions. The calculations could be refined by obtaining specific information from the coating industry. This was in the course of the risk assessment not possible. In such cases it is recommended in the TGD to use the default emission tables of the TGD (EC, 2003a). The values derived from these tables are used in the risk characterisation.

3.1.6 Terrestrial compartment

A default calculation for the local soil concentration is performed, assuming a TCEP deposition from the generic paint formulation site as well as sewage sludge application due to sludge originating from the same site, representing the highest local TCEP concentration in the hydrosphere. This scenario is considered to represent a realistic worst case for the local terrestrial assessment. The following input data are used:

$DEPtotal_{ann}$ = 1.74 • 10^{-5} mg m^{-2} d^{-1}

Ksoil_water = 3.506

Csludge = 6.51 mg/kg

Kair_water = 1.75 • 10^{-8}

kbiosoil = 6.93 • 10^{-7} d^{-1}

The results for the local soil concentrations are listed below:

<u>exposure of the ecosystem</u>

bulk concentration:	$Clocal_{soil}$	= 0.039 mg/kg
porewater concentration:	$Clocal_{soil\_porew}$	= 0.019 mg/l

<u>exposure of the crops (human consumption)</u>

bulk concentration:	$Clocal_{agr.soil}$	= 0.037 mg/kg
porewater concentration:	$Clocal_{agr.soil\_porew}$	= 0.018 mg/l

<u>exposure of grass (cattle)</u>

bulk concentration:	$Clocal_{grassland}$	= 0.0086 mg/kg
porewater concentration:	$Clocal_{grassland\_porew}$	= 0.0042 mg/l

3.1.7 Non compartment specific exposure relevant to the food chain

Since there is no indication that TCEP may show a bioaccumulation potential, a risk characterization for exposure via the food chain is not necessary.

3.1.8 Regional exposure consideration

The calculations for the regional PECs are performed with SimpleBox 2.0. Since no information on the geographic localisation of the processing sites for TCEP and the respective quantities involved is available, the following assumptions are made:

- The local emissions for the formulation and industrial use of TCEP are summed up and distributed to the regional and continental area in a ratio of 50 % to 50 %. Considering the comparatively low total volume in Europe it can be assumed that only specialised companies at few sites work in this field. The default ratio of 10 % to 90 % is used only for the widely spread releases to the environment (diffuse releases through polymers during service life).

- All waste water is expected to be released via WWTP except for life cycle "diffuse releases from polymers" for which a connection rate to WWTP of 80 % is assumed.

- The diffuse releases from polymers and paints (covering 95 % of the total consumption amount) via migration are not yet included in the default emission tables of the TGD.

  Losses might occur during service life of the products (polymers and paints) and disposal. Difficulties in estimating these losses for TCEP arise due to very limited information regarding the actual use of TCEP in products and their application fields at present. Assumptions are made, however these are open to a certain degree of uncertainty.

  There is a recently revised emission scenario document (ESD) on plastic additives (OECD, 2004). The document gives loss factors for flame retardants. However, these relate to solid flame retardant whereas TCEP is in the liquid state at room temperature. Although not appropriate to assess losses for raw materials' handling and compounding, the ESD is applied to estimate diffuse releases originating from service life and disposal to be used in the regional background concentration.

  The total tonnage of TCEP used in polymers is 947 t/a. This tonnage is split 50:50 one half going into indoor service products and the other half into outdoor service. In a first approach it is further assumed that products have a service lifetime of 10 years.

  The waste could be recycled, remain in the environment, be put on landfill or be incinerated. The ESD gives general figures of disposal percentages for UK in the mid-1990s (OECD, 2004). Accordingly, about 8 % of plastics are recycled, 17 % are burned to generate heat and 75 % are incinerated or disposed of to landfill. Based on estimates for automotive and furniture foams from the draft V6 risk assessment (EC, 2003b) it is

assumed that of these 75 % only 5 % is incinerated, the vast majority (95 %) ends up landfilled.

Organic substances are completely destroyed during incineration. Therefore emissions to water and to air are zero (OECD, 2004). In a first approach, no emissions are expected from the recycling process.

With respect to losses to water from landfill, the ESD states that the maximum potential loss could be calculated from the amount of additive remaining in the plastic at disposal, but that it is very unlikely that this amount would be released. Significant leaching of TCEP could be expected due to high solubility of TCEP (7.82 g/l at 20 °C) and the comparatively low adsorption potential. This assumption is fortified by monitoring data where TCEP was detected in the µg/l range in various ground water samples (see 3.1.2.4).

The Landfill Directive (1999/31/EC) calls for decreasing amounts of waste to be sent to landfill in all EU countries. Waste has to be used for energy recovery as much as possible with another potentially important route in the future being gasification of plastics including PUR (EC, 2006a).

There is a very limited amount of monitoring data on releases of TCEP from landfill sites in the EU (see Chapter 3.1.1.4). The Environment Agency (2005) of England and Wales analysed 22 landfill leachates. TCEP was detected in one out of 56 samples analysed (at a concentration of 0.245 µg/l). Metzger and Möhle (2001) investigated landfill leachates originating from household landfills (3 sites) as well as from one hazardous waste site. Concentrations were up to 0.19 µg/l (household) and 5.56 µg/l (hazardous waste site).

There is currently no guidance in the TGD on how to quantify landfill emissions. The data base on monitoring is too scarce to allow for extrapolation from the measurements to the EU. Emissions due to leaching from landfill are being researched at present but might not be available for some time (Pers comm., 2004).

The total tonnage ending up landfilled can be estimated to < 700 t/a. Although leaching can occur for significant time periods after discharge of the products to landfill site, the impact on the value of $PEC_{regional}$ is expected to be small. For now leaching is not taken into account further in the assessment. If in due time a model becomes available, the issue of leaching from landfill could be reconsidered.

Table 3.9 Loss factors for service life and disposal for use of TCEP in polymers according to OECD (2004)

	% loss to water over lifetime	% loss to air over lifetime
Indoor service, leaching to liquid waste	0.05	
Indoor service, volatility		0.05

Outdoor service, leaching to environment	1.6	
Outdoor service, volatility		0.05
incineration	0	0
landfill	unknown	0

10 % of the total resulting emissions from service life and disposal are used as input for the region.

The possible diffuse releases from service life and waste disposal due to application of TCEP in paints are not considered due to a low total amount of TCEP present (5 t/a in region) and general lack of information.

- TCEP is present as an impurity in the substance V6. Production and use of V6 could therefore lead to environmental releases of TCEP. V6 (CAS: 38051-10-4) is currently undergoing ESR risk assessment (4th priority list, Rapporteur: UK). Since there is only one producer in the EU, the site-specific information is confidential.

  Diffuse releases of TCEP stemming from production, formulation (e.g. materials handling and compounding) and processing (e.g. conversion and moulding) including service life of V6 are estimated using the Emission Scenario Document on Plastic Additives (OECD, 2004). These are used for the calculation of the regional background concentration. Confidential site-specific information was provided to the Rapporteur.

  The release model of V6 containing TCEP as impurity was revised within the revision of the risk assessment of the ESR substance V6 (EC, 2006b). Refer to Chapter 3.1.8.1 for more information.

- No direct release to soil was identified. Diffuse release only occurs as a result of dispersive processes. Release is therefore to be expected as a result of deposition from air and sludge application (see Chapter 3.1.4).

The total environmental releases (continental and regional) are summarized in the following table:

Table 3.10 Total releases of TCEP to different compartments (regional and continental)

field of application	ratio reg./cont.	total release to WWTPs (t/a)	total direct release to the surface water (t/a)	total release into the atmosphere (t/a)
processing of polymers	50/50	0.474	--	0.474
formulation of paints	50/50	0.2	--	0.01
industrial use (processing) of paints	50/50	0.5	--	0
use as intermediate	50/50	1.0	--	5.0×10^{-4}
service life of polymers	10/90	0.189	7.632	0.474
disposal	10/90	0	0	0
regional release from V6 production and use	-	0.451	0.113	0.051
total		**2.814**	**7.745**	**1.010**

The regional PECs resulting from the SimpleBox 2.0 calculations are given below (further details are presented in the Appendix 2):

$PECregional_{water}$ = 0.087 µg/l

$PECregional_{nat\ soil}$ = 0.049 µg/kg_{wwt}

$PECregional_{air}$ = 2.27×10^{-4} ng/m^3

3.1.8.1 Update to release model of V6

TCEP is present as impurity in the ESR substance V6 (4.5 – 7.5 %; Rapporteur: UK/IRL). Production and use of V6 could therefore lead to environmental releases of TCEP which should be added to the regional background for the TCEP assessment.

Much of the V6 life cycle is confidential. In 2003, the Environment Agency made an estimate of the releases of TCEP caused by handling of V6 (Pers. comm., 2003). This estimation used a blanket correction factor to correct for the difference between release rates of "high" and "low" volatility plastic additives, as suggested in the ESD. TCEP is of "high" volatility while V6 is of "low" volatility. The resulting release rates were made available to the Rapporteur

and used in the calculation of the regional background concentration of TCEP (cf. Table 3.10).

In the course of updating the exposure assessment of V6 (agreed at TCNES IV/2006) the release rates from foams had been subject to modelling and measurements. The physicochemical properties of TCEP are closer to TCPP than to V6. New release rates were applied using percentage rates of release of TCPP from equivalent life cycle stages. The resulting releases are shown in Table 3.11. It has to be noted that a change in the approach to modelling of waste remaining in the environment resulted in an overall increase in estimated release rate to air.

Table 3.11 Comparison of releases of TCEP from V6 handling between 2007 model and 2003 estimation

	to WWTPs (t/a)	**to surface water (directly) (t/a)**	**to atmosphere (t/a)**
regional release from V6 production and use (2007)	0.003	7.3×10^{-4}	0.089
total release (2007)	2.366	7.633	1.048
regional release from V6 production and use (2003)	*0.451*	*0.113*	*0.051*
total release (2003)	*2.814*	*7.745*	*1.010*

The respective regional PECs resulting from the SimpleBox 2.0 calculations using the total releases are given below (Table 3.12):

Table 3.12 Comparison of PECregional for various compartments using different V6 release rates

	2007 model	**2003 estimation**
$PEC_{regional_{water}}$	**0.071 µg/l**	**0.087 µg/l**
$PEC_{regional_{nat\ soil}}$	**0.055 µg/kg_{wwt}**	**0.049 µg/kg_{wwt}**
$PEC_{regional_{air}}$	**2.52×10^{-4} ng/m^3**	**2.27×10^{-4} ng/m^3**

It can be noted that regional PEC for water from 2007 model is smaller than from 2003 estimation. Both regional 2007-PECs for air and soil are higher than 2003-PECs. However, this change has no significance for the resulting local PECs and the consequent PEC/PNEC ratios (cf. Chapter 3.3).

The refinements to the exposure model of V6 have changed the release rates of TCEP from V6 handling, and consequently the total release rates and the various PECregional. Although more accurate these changes have no significance for the PEC/PNEC ratios. For reasons of simplicity the PECregional as calculated from 2003 estimation are used in the risk assessment.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment

The available information about ecotoxic effects produced in various tests is summarized in the Table 3.11 below. The effects recorded refer almost exclusively to short-term exposure. All effect values refer to nominal concentrations. Only freshwater species have been tested.

3.2.1.1 Toxicity to algae

Noteworthy are striking differences between the NOEC/EC_{50} values determined in the tests conducted with *Scenedesmus subspicatus*.

An examination of the respective test protocols failed to establish the reasons for such large discrepancies. The differing algal strains and nutrient media used cannot be regarded as a plausible primary cause. The only difference between the test conditions of the two tests with *Scenedesmus subspicatus* was a difference in pH of about 0.5 unit. The test by Kühn et al. (1989) used at test start a pH of 8, while the study by Hoechst (1988) employed a pH of 7.5. However, it is not assumed that this difference in pH has such an influence on the test results.

As in the study by Kühn et al. (1989) a pH increase by 1.4 units (from 8.0 to 9.4) occurs between day 2 and 3 of exposure that may have influenced the toxicity, even if the validity criterion is fulfilled, it is proposed by Nyholm & Kusk (2003) to use the 48h-effect values from this study instead of the 72 h-values.

The test results cited in Akzo (1993) cannot be validated as neither a test report nor a reference is available. As one of the given values is in agreement with the results found by Kühn et al. (1989), it is assumed that the same test result is described.

In the 96 h test conducted with another green alga (*Selenastrum capricornutum*), considerable differences between the results of the range-finding test with no growth inhibition up to 100 mg/l and the outcome of the definitive test (NOEC = 5 mg/l) were found. In addition, a comparison of the growth characteristics given for both preliminary and definitive test reveals significantly stronger growth even in the controls in the former test, supplemented here by slight promotion of cell multiplication (compared to controls) at all concentrations tested (range: 0.1 - 100 mg/l). The authors cite the noticed instability of the TCEP dispersion as potential reason for the discrepancies in the effect values for both tests (Akzo, 1992). However, this observed instability cannot be explained by the physicochemical properties of the substance nor by any degradation mechanism.

3.2.1.2 Toxicity to aquatic invertebrates

On the whole the few available data indicate modest to low toxicity to the representatives of this group (although 48 h values for *Daphnia* are lacking) under conditions of short-term exposure. The results of the 7 d investigation with planarians collected in an unpolluted river should be considered as merely indicative values: Yoshioka et al. (1986) concluded from their correlation analysis including several test methods with various test species that the additional stress exerted by head cutting may influence the effect values. In this sense, planarian sensitivity may be lower than indicated by this head and eye regeneration study.

Table 3.11: Data on ecotoxic effects of TCEP on aquatic plants (algae), invertebrates and fish

Taxon. group	**Species**	**Exposure duration**	**Endpoint**	**Effect value (mg/l)**	**Reference/annotations**
Green algae	*Scenedesmus subspicatus*	48 h	E_bC_{10} E_rC_{10} E_bC_{50} E_rC_{50}	0.29 0.65 2.0 5.0	Kühn et al., 1989 (WaBoLu res. Report N° 106 03 052/01)
		72 h	E_bC_{10} E_rC_{10} E_bC_{50} E_rC_{50}	0.2 0.55 1.1 3.6	
		96 h	E_bC_{10} EbC_{50}	0.3 1.2	
		72 h	NOEC E_bC_{50}	100 271, 278	Hoechst AG, 1988 Report N° OEK 88/082 E) two separate tests
			E_rC_{10} E_rC_{50}	148 522	Recalculation by Nyholm & Kusk (2003) from raw data
			EC_{10} EC_{50}	15.9 67.9	cited in Akzo, 1993, test report or reference not available (it is only stated that the value is from Akzo chemical database, containing various sources)
		72 h	EC_{10} EC_{50}	0.2 1.1	cited in Akzo, 1993, test report or reference not available (it is only stated that the value is from Akzo chemical database, containing various sources)
	Selenastrum capricornutum	96 h	NOEC LOEC E_bC_{50} E_rC_{50}	5 15 38 117	Akzo Chemicals, 1992 (CRL report F 92 016) in range finding test NOEC = 100 mg/l
Inverte-brates	Crustacea: *Daphnia magna*	24 h	EC_0 EC_{50} EC_{100}	100 340 1000	Hoechst AG, 1989
		24 h	EC_0 EC_{50}	186 451	Kühn et al., 1989 (WaBoLu res. Report

		21 d	NOEC	13	N° 106 03 052/01) E = reproduction rate
		24 h	EC_{50}	235	Bayer AG, 1991
	Moina macrocopa	3 h	LC_{50}	1000	Yoshioka et al., 1986
	Planaria (flatworms): *Dugesia japonica*	7 d	EC_{50}/ LC_{50}	159	Yoshioka et al., 1986 head regeneration and mortality test

Table 3.11: continue

Taxon. Group	**Species**	**Exposure duration**	**Endpoint**	**Effect value (mg/l)**	**Reference/annotations**
Fish	*Oncorhynchus mykiss*	96 h	LC_0 LC_{50} LC_{100}	100 249 400	Akzo Chemicals, 1990b LSR report N° 90/AKL 021/0161)
	Oryzias latipes	48 h	LC_{50}	300	MITI, 1992
		96 h	LC_{50}	210	Sasaki et al., 1981
		96 h	LC_{50}	170	Yoshioka and Ose, 1993
		48 h	LC_{50}	230 (10 °C) 190 (20 °C) 66 (30 °C)	Tsuji et al., 1986
	Leuciscus idus melanotus	48 h 2 h	LC_0 EC_0 LC_{50} LC_{100}	ca. 100 10 ca. 200 300	Hoechst AG, 1978 (test report 6/78)
	Carassius auratus	96 h	LC_{50}	90	Sasaki et al., 1981
		7 d	EC_0/LC_0	5	Eldefrawi et al., 1977 (only one concentration tested)
	<u>dietary toxicity:</u> *Cyprinus carpio*	6 d	LD_0	up to 156 mg/kg food	Loeb and Kelly, 1963 (US Fish and Wildlife Serv.)

3.2.1.3 Toxicity to fish

The data on the impact of short-term exposure indicate modest to low acute toxicity. As symptoms of progressive poisoning hyperventilation, loss of coordination in swimming, darkening pigmentation and apathy are mentioned in test protocols. At concentrations higher than 200 mg/l, 100 % mortality normally occurred within the first day of exposure. Sasaki et al. (1981) reported a deformation of the spinal column in 3 of 12 killifish after exposure to 200 mg/l for 24 - 72 h. Under these exposure conditions, protrusion of eyes was observed, too.

The 48 h LC_{50} values for killifish determined by Tsuji et al. (1986) in a static system illustrate the increase in toxic action resulting from rising temperature.

With regard to the few LC_0/NOEC values reported, some comments in the test protocols provide supplementary information about the range of the acute no-effect level in fish:

- in the 96 h test with juvenile trout (Akzo, 1990b) during the preliminary range finding investigation hyperventilation and darkening pigmentation were observed even at the lowest concentration tested (10 mg/l). These findings contrast considerably with the NOEC of 50 mg/l resulting from the definitive test. The differences in the results may be due to incomplete solution of TCEP as observed in the latter test only. However, this incomplete solution cannot be explained by the physicochemical properties of the test substance.

- during the 48 h test with golden orfe (Hoechst,1978) increasing disturbances in swimming at concentrations higher than 10 mg/l were recorded (at 25 mg/l in one, at 50 mg/l in two of 10 fish).

These auxiliary data appear to indicate that TCEP concentrations around 10 mg/l may represent a kind of threshold level for sublethal effects in sensitive fish (like trout) under conditions of short-term exposure.

The 7 d EC_0/LC_0 (5 mg/l) for goldfish determined by Eldefrawi et al. (1977) in a simple tank experiment is restricted in its indicative value regarding the NOEL to concentrations of 5 mg/l: since only one concentration (5 mg/l) was tested, higher EC_0 and LC_0 values cannot be excluded.

As mentioned in Chapter 3.1.1, TCEP bioconcentration by fish is low (Sasaki et al. 1981, 1982). Uptake and elimination take place rapidly as shown by a biological half-life value of 0.7 h in killifish (Sasaki et al., 1982). At test concentrations of 1 - 3 mg/l, the maximum bioconcentration ratio in killifish (1.2 - 1.4) was reached within the first day and remained practically constant until cessation of exposure after further 10 days. The former feature may at least partly explain the above mentioned early manifestation of mortality in fish lethality tests at higher concentration levels.

The analytical monitoring of TCEP carried out by Sasaki et al. (1982) did not trace metabolites of the test substance. Supplementary information about the potential extent of metabolic conversion was provided by Sasaki et al. (1985). They reported that in contrast to certain other phosphoric triesters the compound was only slightly affected when incubated with liver microsomes of goldfish in the presence of NADPH.

3.2.1.4 Toxicity to microorganisms

In a respiration inhibition test according to OECD guideline 209 activated sludge of a predominantly domestic sewage treatment plant was exposed to TCEP for 3 h. The EC_{20}, EC_{50} and EC_{90} were 1.4 g/l, 3.2 g/l and 7.8 g/l, respectively (Akzo, 1990c).

The aerobic degradation of synthetic sewage by industrial activated sludge was inhibited at concentrations above 500 mg/l, up to 1000 mg/l inhibition was compensated after 1 day adaptation; below 500 mg/l degradation was rather enhanced (Hoechst, 1978).

In an inhibition test applying the ETAD fermentation tube method with anaerobic microorganisms gas evolution was inhibited at concentrations above 250 mg/l (Hoechst, 1978). In a second assay an EC_0 of 1500 mg/l after 24 h was reported (Hoechst, 1985). However, with the same method EC_{50} values of 10 - 100 mg/l were cited in an EU safety data sheet (Hoechst, 1994).

3.2.1.5 Determination of the $PNEC_{aqua}$

Despite remaining problems regarding the plausibility of widely differing effect values resulting from growth inhibition tests with algae, the lowest effect figures available refer to this group. As the available studies are regarded as valid and no reason for the conflicting results can be given, it is proposed to use the lowest effect value based on growth rate for the derivation of the PNECaqua. As explained above, the 48h-values are preferred to the 72h-values. Therefore, the 48h-ErC_{10} of 0.65 mg/l found by Kühn et al. for *Scenedesmus subspicatus* is used as basic value. Long-term tests with species from two trophic levels are available. Therefore an assessment factor of 50 can be regarded as suitable. However, as from the effect values for *Scenedesmus subspicatus* found by Kühn et al. it can be concluded, that algae are the most sensitive species to TCEP (EC50-value is a factor of 18 to 90 lower than EC50/LC50 values from fish and daphnids found in short-term tests), and it is therefore not expected that in a long-term test with fish an effect value below 0.65 mg/l will be found, an assessment factor of 10 is justified according to the TGD:

$$\mathbf{PNEC_{aqua}} = 0.65 \text{ mg/l} / 10 = \mathbf{65\ \mu g/l}$$

3.2.1.6 Determination of the $PNEC_{wwtp}$

For the effects assessment for microorganisms in sewage treatment plants the $PNEC_{wwtp}$ is calculated by applying an assessment factor of 100 on the EC_{50} from the OECD 209 respiration inhibition test (3.2 g/l) according to the TGD:

$$\mathbf{PNEC_{wwtp} = 32\ mg/l}$$

3.2.1.7 Sediment

No information about TCEP effects on sediment organisms could be found.

Consequently, only a provisional $PNEC_{sed}$ can be determined based on equilibrium partitioning according to TGD using a $K_{susp\text{-}water}$ of 3.655:

$$\mathbf{PNEC_{sed}} = \frac{3.655 \bullet 0.065 \bullet 1000}{1150} = \mathbf{0.2\ mg/kg\ ww}$$

3.2.2 Atmosphere

No ecotoxicological data are available for this environmental compartment.

3.2.3 Terrestrial compartment

The taxon-related description and discussion of reported effects include soil organisms and species/groups inhabiting other terrestrial substrates.

With regard to soil, all relevant data available were generated by Römbke et al. (1995).

3.2.3.1 Toxicity to higher plants

The following results have been obtained for *Avena sativa* (oat) in a plant growth test conducted according to OECD 208 (slightly modified):

14 (16) d NOEC = 10 mg/kg (dw)

EC_{50} = 64 mg/kg (dw)

Römbke et al. (1995) mention that due to lacking data homogeneity, the estimated NOEC of 10 mg/kg could not be verified statistically.

At the lowest concentration within the range tested (1 - 1000 mg/kg) necrosis and chlorosis at leaf tips were observed. Though indicating that the overall no-effect-level for the ecotoxicological impact of TCEP on growth-related processes lies below 1 mg/kg, in the respective evaluation it has to be taken into account that at concentrations up to 10 mg/kg (dw) *Avena* plants were evidently able to recover from those injuries before the end of the test. The rapid loss of recovery potential at increasing concentrations is shown by a 76 % growth inhibition reported for 100 mg/kg (dw).

In contrast to the data for 14 (16) d exposure, in the preceding seed germination and seedling emergence test lasting 48 h no inhibition could be established at concentrations up to 1000 mg/kg (dw).

3.2.3.2 Toxicity to invertebrates

No mortality could be observed at concentrations of up to 1000 mg/kg in a 14 d earthworm toxicity test conducted according to a BBA guideline which (despite very slight

modifications) corresponds to OECD 207. However, at that maximum concentration a significant weight reduction (> 10 %, compared to controls) in *Eisenia andrei* was determined. As NOEC for this endpoint a value of 580 mg/kg (resulting from the chosen spacing factor of $\sqrt{3}$) was reported (Römbke et al., 1995).

Among arthropods, a few insect species and a spider were investigated for their TCEP susceptibility:

The springtail *Folsomia candida* (3. - 5. larval stage at test start) was exposed in a 28 d reproduction test in accordance with a BBA guideline (Riepert, 1991) which corresponded to the analogous ISO draft. The following results were obtained (values in mg/kg (dw)):

mortality (adults):	LC_{10} = 19.3
	LC_{50} = 66.5
reproduction rate:	EC_{10} = 44.6
	EC_{50} = 131.9

The authors reported that a 30 % reduction in the reproduction rate was judged to be not statistically significant due to high rate variability observed when repeating the test, in contrast to the respective value of almost 90 % obtained for 125 mg/kg.

In the preceding 24 h test conducted with adult *Folsomia* specimens ($\geq$ 3 mm in length) first signs of intoxication had been observed only at the highest concentration tested (1000 mg/kg).

As further insect species the carnivorous carabid beetle *Poecilus cupreus* representing the epigean fauna was subjected to a bait-lamina test (BBA guideline VI, 23-2.1.8, 1991). In this procedure, counting of traces of feeding activity on fly pupae *(Calliophora)* on soil (quartz sand, 70 % of maximum water capacity) is carried out. Groups of adults were exposed to 5 mg/kg (dw) for two weeks. No mortality or any behavioural effects were observed for two weeks in 5 replicates, but a mean reduction of 28 % in the feeding rate was determined. This effect, though not designating the chosen TCEP concentration as precise LOEC, may be seen as quite indicative: according to an EC proposal (1991) a rate of > 30 % should be used as qualifying criterion within the regulation process for plant protection agents.

Supplementary information about TCEP toxicity to insects refers to species which are not or only occasionally exposed to pollutants in soil: Ludvik and Decker (1947) reported a 48 h mortality rate of 51.3 % in aphids *(Myzus persicae)* after spraying with 2000 mg/l. No mortality was observed after 72 h when adult houseflies (*Musca domestica*) were fed a mixture of 1 % TCEP in sugar and dried milk. Topical application in this species led to the same result (Eldefrawi et al., 1977).

On the whole, the cited results point to low TCEP impact on insects under conditions of 1 - 3 d exposure. Regarding the mechanism of action, this view is affirmed in part by research of Eldefrawi et al. (1977) in which TCEP turned out to be a weak enzyme inhibitor in the midgut system of larval armyworm *(Spodoptera sp.)*.

The only available information on TCEP toxicity to spiders was provided by Römbke et al. (1995) who conducted a substrate contact test according to a respective BBA guideline (draft, 1992). Subadult females of the epigean genus *Pardosa* were exposed to 5 mg/kg (dw) contained in moistened quartz sand for two weeks. A 14 d mortality rate of 41.7 % was established, supplemented by behavioural effects (disturbances in locomotion) starting on day 9 of exposure. No effect on the feeding rate could be determined. The authors mentioned that the cited mortality rate due to the low number of test organisms (groups of 4 spiders in 3 replicates) could not be statistically verified. However, the observed sublethal effect on the motility should be evaluated as being ecotoxicologically relevant.

3.2.3.3 Toxicity to soil microorganisms

The impact of TCEP on soil microflora was investigated in two natural soils under aerobic conditions with 5 mg/kg and 50 mg/kg soil, resp., for 28 days (Römbke et al., 1995). In the sandy soil (C_{org} < 1 %) with 5 mg/kg the dehydrogenase activity, after an initial increase of 21 %, was inhibited by 15 % after 28 days. With 50 mg/kg, after an initial increase of 16 %, the inhibition increased up to 42 %. In the loamy soil (C_{org} = 2.2 %) only the high concentration caused an inhibition > 15 % (20 % inhibition after 28 d).

3.2.3.4 Toxicity to birds

Sprague et al. (1981) tested TCEP for delayed neurotoxicity on 12- to 14-month-old white Leghorn hens. 24 h after a single oral application of undiluted TCEP at a dose of 10 ml/kg (= 14.2 g/kg), or undiluted corn oil (2 ml/kg) as a control, they investigated the inhibition of a protein processing esterase activity in vitro (referred to as "neurotoxic esterase"). Marked inhibition of this enzyme (> 75 %) is regarded as an indicator of delayed neurotoxicity.

A 30 % inhibition of this enzyme was observed, which was statistically significant ($p < 0.05$). At the same time the plasma-cholinesterase activity was inhibited by 87.1 % ($p < 0.05$). Since the inhibition of the "neurotoxic esterase" of the brain was not greater than 75 % the authors postulated that TCEP cannot cause delayed neurotoxicity.

This view was corroborated in a second study in which 10 ml/kg (= 14.2 g/kg) undiluted TCEP or 10 ml/kg undiluted corn oil, were administered orally twice, with an interval of 3 weeks. During the three weeks after the last application, and at the end of this period, no group showed any indications of delayed neurotoxicity either behaviourally or histopathologically. In particular, no axonal degeneration was observed.

Treatment with TCEP did, nevertheless, coincide with a decrease in body weight, a transient decrease in food uptake immediately after the applications, a cessation of egg production, and the induction of moulting. Four out of 18 animals died during the study.

In another study on hens, TCEP again failed to show neurotoxic effects following oral application of 2.5 g/kg or intraperitoneal application of 1.0 g/kg body weight (Bayer, 1986).

3.2.3.5 $PNEC_{soil}$ calculation

On the basis of the various effect values reported (supplemented by information of merely indicative value) higher plants may be regarded as being somewhat more sensitive to TCEP than susceptible invertebrates. Regarding invertebrates, the available information points to notably higher susceptibility of arthropods compared to earthworms. The poor data on *Pardosa* do not allow to draw further detailed conclusions with respect to a higher sensitivity of insects or spiders. On the whole, the data point to similar susceptibility.

Long-term tests are available for springtails and soil microorganisms. No significant differences in sensitivity between *Folsomia* and bacteria can be derived from the respective test results for 28 d exposure.

However, since the available information on *Folsomia* covers a broader spectrum of effects, the lowest effect value reported for this species is chosen as reference value for the $PNEC_{soil}$ derivation (28 d LC_{10} = 19.3 mg/kg dw for adults, assumed to represent a NOEC).

According to the TGD an assessment factor of 50 has to be applied to this value.

$PNEC_{soil}$ = 19.3 mg/kg (dry weight)/ 50 = 0.386 mg/kg (dry weight)

$PNEC_{soil}$ = 0.341 mg/kg (wet weight)

3.2.4 Non compartment specific effects relevant to the food chain (secondary poisoning)

Since TCEP does not possess a bioaccumulation potential, the derivation of a $PNEC_{oral}$ is not necessary.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

(incl. sediment)

Waste water treatment plants

The highest discharge concentration for waste water treatment plants was calculated as **0.370 mg/l** for the industrial use of paints and varnishes. Generic model is used here for the calculation of the $Clocal_{effl}$. No specific information is available for this area of use of the substance or for the exposure in the environment. Consequently, standard scenarios had to be used for the calculation of the concentration in the waste water treatment plant.

Taking into consideration a **$PNEC_{WWTP}$** of **32 mg/l**, a $Clocal_{effl}$/PNEC ratio of **0.01** results for the industrial use of paints and varnishes. Since the $Clocal_{effl}$/PNEC ratio **< 1**, there is no risk expected to the microorganism population in the WWTP.

Conclusion (ii)

Aquatic environments

The PEC/PNEC ratios for all of the areas of production, formulation and processing are summarized in the following table. The ratios are calculated using a $PECregional_{water}$ of 8.71 x 10^{-5} mg/l and a **PNEC** of **65 µg/l**.

Table 3.12 Risk characterisation for aquatic compartment

Company/area of use	PEC_{local} (µg/l)	$PEC/PNEC_{aqua}$
Production and processing*	28.9	0.44
Processing only	4.20	0.06
Processing of polymers	6.25	0.10
Formulation of paints and varnishes	16.52	0.25
Processing of paints and varnishes (industrial use)	37.1	0.57

* for information only, since no production of TCEP in EU anymore

PEC/PNEC < 1 could be derived for all life cycle steps using generic calculations.

Conclusion (ii)

Sediment

No effect values on sediment organisms could be found. This allowed only for the derivation of a provisional $PNEC_{sed}$ using the equilibrium partitioning method.

A $PEClocal_{sed}$ based on the $PEClocal_{water}$ was calculated in Chapter 3.1.1.3, but hereof no information beyond those obtained from the risk characterisation for the water phase can be derived.

Some monitoring data from sediments of different rivers in Germany are available. However, the geographical area concerned is small and no information regarding the representativeness of the data is available. The data basis is therefore regarded not sufficient for risk characterisation.

3.3.2 Atmosphere

Due to the atmospheric half-life ($t_{1/2}$ = 17.5 h), abiotic effects on the atmosphere, such as global warming and ozone depletion, are not to be expected in connection with TCEP. The highest calculated air concentration is around 0.035 µg/m^3 for processing of polymers. Since no data are available on the ecotoxicological effect of the substance in connection with this environmental compartment, it is not possible to perform a quantitative assessment of this environmental compartment. On the basis of the available information on the substance, further testing seems not necessary.

Conclusion (ii)

3.3.3 Terrestrial compartment

Releases into the terrestrial compartment as a result of deposition from the atmosphere and sewage sludge application are to be expected. The highest soil concentration for TCEP amounting to 0.039 mg/kg (wet weight) results from the formulation of paints and varnishes giving a PEC_{soil} of **0.039 mg/kg** (wet weight).

Based on the $PNEC_{soil}$ of **341 µg/kg** (wet weight) a PEC/PNEC ratio of **0.12** is calculated.

There is at present no indication of a risk to the local terrestrial environment.

Conclusion (ii)

3.3.4 PBT-assessment

The following table shows the criteria as defined in the TGD to identify PBT/vPvB substances, and the values relevant for TCEP. The description of the relevant tests can be found in Chapter 3 (**P** and **B**) and in Chapter 3.2 (**T**).

Table 3.13 Data for TCEP and PBT/vPvB criteria according to TGD

Criterion	PBT-criteria	vPvB-criteria	TCEP
P	Half-life > 60 d in marine water or > 40 d in freshwater or half-life > 180 d in marine sediment or > 120 d in freshwater sediment	Half-life > 60 d in marine- or freshwater or half-life > 180 d in marine or freshwater sediment	non biodegradable (surface water) DT_{50} (soil): 167 d
B	BCF > 2000	BCF > 5000	BCF (fish): < 1.2 – 5.1
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	E_rC_{10} (algae): 0.65 mg/l Carcinogenic Cat. 2 Toxic for Reproduction Cat. 2

TCEP has to be considered as non biodegradable (DT_{50}: ∞ days). An investigation on primary degradation of TCEP in soil showed a half life of 167 days.

The highest measured BCF in fish are <1.2 – 5.1.

The lowest long-term effect value of 0.65 mg/l was found for *Scenedesmus subspicatus*.

TCEP has been proposed to be classified as Carcinogenic (Cat. 2). There is evidence of chronic toxicity (T, R45).

It can be concluded that TCEP meets the **P/vP**- and the **T**-criteria. The **B**-criteria is not fulfilled. Overall TCEP does not meet the PBT criteria.

There is not enough information available to exclude the possibility of sites being located at the sea. TCEP is not degradable and shows limited sorption (98.6 % released to water from WWTP). The concentration in seawater can be estimated to be about 10 % of that in freshwater. As the marine PNEC will be 10 % of the freshwater PNEC, the overall marine PEC/PNEC ratios will be similar to those for freshwater. Due to the low BCF values bioaccumulation is not expected and the assessment of secondary poisoning is not considered necessary. In view of all arguments above, there is no need for a marine risk assessment.

3.3.5 Non compartment specific effects relevant to the food chain (secondary poisoning)

Since there is no indication of bioaccumulation of TCEP, a risk characterisation for exposure via the food chain is not necessary.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

In the EU, Poland is at present the only country producing Tris(2-chlorethyl)phosphate (TCEP, amount and use pattern confidential). The production and import of TCEP has declined during the past years. In the year 2001, there was no production within the EU, but marketing of TCEP-containing products was and is still relevant (app.1000 t of TCEP in 2002).

The main use of TCEP is the production of unsaturated polyester resins. One company supplied quantitative information about the application areas of their sales in 2001:

Unsaturated polyester resins	83 %
Acrylic resins	2 %
Adhesives	5 %
Coatings (wood and roofings)	<1 %
Polyurethane	<1 %
Others (not known)	9 %
Cellulose acetate	1 %
Textile	0 %

The company states, that the unknown applications (2.3 % corresponding to 10 – 15 tons) might eventually be used in paints and varnishes. Three companies give information on the use of TCEP in

Marine and protective coatings
Industrial paints
Fire retarding lacquers and paints (2.5 – 10 %)
Plasticiser with flame retarding properties in water based intumescent material (up to 7%)
Polyurethane industry
Flame retardant for flexible slabstock polyether and moulded foams (automotive industry, furniture industry).

According to information from 1987, the main industrial branches using formed products containing TCEP as a flame-retardant plasticizer are the furniture, the textile and the building industry (roof insulation); TCEP is also used in the manufacture of cars, railways and aeroplanes (GDCh, 1987).

Information on the concentrations of TCEP are not the same: due to information from two producers the concentration of TCEP in products amounts to 5 - 12 % (w/w). In addition in one product (cellulose acetate) a concentration of up to 70 % TCEP is possible. According to literature data concentrations up to 40 % are used.

Industry informed the Rapporteur, that less than 10 t/y TCEP are applied in paints.

According to information from the producers, since its classification as a carcinogenic (category 3 a), the use of TCEP in polyurethane foams has declined to a large extent and is currently limited to special products.

For further detailed information on the uses of TCEP see chapter 2.

For workers the inhalation and dermal exposure routes are the most likely.

4.1.1.2 Occupational exposure

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90^{th} or 95^{th} percentiles are derived as representing reasonable worst case situations. For the purpose of exposure assessment only data measured later than 1990, if available, are taken. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data is not available, model estimates are taken forward to the risk characterisation. Short term exposure levels are given on the basis of measurement data. In lack of such data short term exposure levels are only calculated when they are needed for the risk characterisation. This is only rarely the case. The procedure is applied because there is no common understanding on deriving short term exposure levels on the basis of modelled 8 h TWA.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:
Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.
Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.
There is an agreement between the EU member states, within the framework of existing substance, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided, that for a certain scenario gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as criteria. For most down stream uses it is commonly known, that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since often quantitative information on dermal exposure is not available, the EASE model is used for assessing dermal exposure, at the most.

Industrial activities using TCEP present opportunities for exposure. Exposure ranges depend on the physico-chemical properties of the substance, the particular operation and the risk reduction measures in use. TCEP is a liquid with a vapour pressure of 0.00114 Pa at 20 °C (extrapolated). Inhalation exposure is to be expected if processes are performed at elevated temperatures or if dusts containing TCEP are formed. Industry states that processes at

elevated temperature do not occur because of the degradation of TCEP at elevated temperatures and that the substance or products containing the substance do not occur in a powdery state.

The following scenarios are regarded to be relevant for occupational exposure:

Scenario 1: Production of TCEP

Scenario 2: Use of TCEP at ambient temperatures for the production of polymers and formulations

Scenario 3: Use of formulations and products containing TCEP

The flame retardant TCEP is physically bound within the polymer matrix and, therefore, TCEP could migrate to the surface especially during processing steps especially those performed at high temperatures. Therefore release of TCEP from plastic products may be a potential way of exposure. In this, it should be considered, that not only plastic products produced in Europe but also products imported are concerned. The very low vapour pressure at room temperature and the high molecular weight lead to negligible migration. A prediction of consumer exposure caused by the release of TCEP from plastic products is made in section 4.1.1.3 “consumer exposure”. In conclusion, exposure to TCEP during subsequent use of flame retarded equipment is likely to be negligible.

Since no information on exposure levels are available, the assessment of inhalation and dermal exposure is based on model estimates (according to the EASE model). Because the composition of the applied formulations (paints, adhesives etc.) is generally not known, it is not possible to correct the inhalation EASE estimate for the pure substance in consideration of the percentage of TCEP.

The number of exposed workers is not known for any of these scenarios.

OELs are not established in the EU.

Recycling:

There is no information concerning the exposure during recycling of plastic waste. Generally, the recycling of halogenated flame retardants is problematic, because of the possible release of halogenated compounds into the environment. There are two possibilities in the work up of plastic waste: thermic and shredding. It is supposed that mixtures of different plastics are recycled together.

4.1.1.2.1 Production of TCEP (scenario 1)

At present, TCEP is not produced in the EU but in the new EU member state Poland. Limited confidential information is available from the polish company. To some extend, information provided by companies which terminated the production is taken into account.

TCEP is produced by the catalytic addition of ethylene oxide and phoshoryl chloride in closed systems. The pure product is obtained after cleaning steps, removal of the catalyst and drying in vacuum. The production is described to be continuously or batch-wise. The liquid product is filled in tanks via closed pipelines or in drums using local exhaust ventilation (Hoechst, 1994).

For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached, e.g. during filling. Exposure may occur during coupling and uncoupling of transfer lines, drumming, cleaning, maintenance, repair works and the taking of process samples. It is assumed, that such activities are predominantly performed with local exhaust ventilation (LEV). According to information from the producers, personal protective equipment (gloves, eye protection and protective clothes) are worn.

Inhalation exposure

Workplace measurements

A limited number of confidential workplace measurement results were provided by one producer, which terminated the production.

EASE estimation

EASE for Windows 2.0, Aug. 1997

EASE estimation for the production and further processing of TCEP in the large-scale chemical industry:

Input parameters: T = 20 °C, closed system, significant breaching, LEV present

(vapour pressure < 1 Pa)

Level of exposure: 0 – 1.2 mg/m^3 (0 – 0.1 ml/m^3).

Due to the low vapour pressure of TCEP, the same exposure level is predicted for workplaces without LEV.

Conclusions

Since representative workplace measurement results are not available, exposure levels of 0 – 1.2 mg/m^3 (8-h TWA) predicted by the EASE model are used to assess the risks of inhalative exposure. 1.2 mg/m^3 should be taken as representing the reasonable worst case situation.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

The assessed exposure level (incl. duration of exposure) is representative for the Polish company.

Dermal exposure

When producing and further processing dermal exposure could occur during activities like drumming, coupling and uncoupling transfer lines, sampling, cleaning, maintenance and repair work. For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows:

Input parameters:	Non dispersive use, direct handling, intermittent
Level of exposure:	0.1 – 1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (palms of hands) the model yields an exposure level of 42 - 420 mg/person/day. For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted. The extent of protection of the personal protective equipment (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of the substance. Beside this fundamental suitability, the level of protection depends on the real working conditions, i.e. the working behavior, the information of the workers etc.

Three producers and importers submitted information about the used glove material (rubber). But there is a lack of information with regard to the suitability of the recommended materials. For the use of unsuitable gloves, dermal exposure is assessed as worst case estimation applying the EASE model, estimating a dermal exposure level for immediate dermal contact without gloves. It is not possible to consider the limited protection provided by unsuitable glove material.

Since the suitability of the gloves cannot be presupposed, the model estimates of 42 – 420 mg/person/day for the unprotected worker should be taken.

Conclusions

For assessing the health risks from daily dermal exposure in the area of production and further processing (scenario 1), an exposure level of 42 – 420 mg/person/day should be taken. This exposure assessment is based on information provided by the companies, that unsuitable gloves are worn and takes into account the possible dermal exposure under actual workplace conditions.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Use of TCEP at ambient temperatures for the production of polymers and formulations (scenario 2)

TCEP is used as a plasticizer in different formulations (e.g. paints, lacquers) containing cellulose derivatives, polyvinyl acetate etc. (Baumann, 1997; Biethan et al., 1979) in different resins (for glues and adhesives) and in polymers (polyurethane, cellulose acetate). According to the available information the concentration of TCEP in these formulations amounts to 5 to 16 %. In the starting polymer mixture (liquid, dispersion) higher concentrations of up to 40 % are possible. It is to be expected, that the formulation processes often have several steps in common, e.g. transporting and filling, mixing on site with additives and the base polymers or

resins, adjusting and filling the products into drums or other containers. Information on the formulation of products containing TCEP is provided by one company (Hoechst, 1994).

There is no detailed information which processing steps are applied in case of TCEP. Therefore, only a very general description can be given. According to information from the representative company, TCEP is neither handled in powdery formulations nor at elevated temperature.

According to information provided by one company incompany transfer of TCEP occurs via closed pipelines. TCEP is brought to the customer sites mainly in tanks, to a less extent in drums. The substance is filled into storage tanks equipped with fixed pipelines to the location of further processing. The further processing is performed in closed systems and filling of the final product (here containing 16 % TCEP) is done with a local exhaust ventilation being present. Duration and frequency of exposure are assumed to be full shift and daily, although transfer at beginning of the process and drumming may be done only during part of the day, especially if production is discontinuously. In this case, duration of inhalation and dermal exposure may be shorter than the shift length.

Inhalation Exposure

Workplace measurements

Workplace measurement results are not available.

EASE estimation

Input parameters: T = 20°C, closed system breached or non-dispersive use (vapour pressure < 1 Pa).

Level of exposure: 0 – 0.1 ppm (0 – 1.2 mg/m³)

Conclusions

Because no monitoring data are available, the EASE model was used to estimate the potential exposure at the workplace. In general for vapour pressures below 1 Pa the result of the estimate is 0 – 1.2 mg/m^3 (0 – 0.1 ppm), independent of the use pattern “closed system” (with the possibility to be breached), “non-dispersive use”, or “wide dispersive use”. It is to be assumed that the substance is processed daily, so the duration and the frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

Dermal exposure

For the further processing of TCEP in the production of polymers and of formulations, (e.g. plastics manufacturing industry) it cannot be excluded that gloves are not regularly worn and

that immediate dermal contacts occur during activities like drumming, filling, cleaning, repair and maintenance. The corresponding exposure for the unprotected worker is assessed in application of the EASE model:

Input parameters:	Direct handling, non dispersive use, intermittent
Exposure levels:	0.1 - 1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (palms of two hands) the exposure level of daily dermal exposure amounts to 42 - 420 mg/person/day.

Conclusions

For assessing the health risks of daily dermal exposure in the area of production of polymers and formiulations (scenario 2), an exposure level of 42 – 420 mg/person/day should be taken. This exposure assessment is based on the assumption that suitable gloves are not worn. It cannot be presupposed, that eye protection is regularly used.

4.1.1.2.3 Use of formulations and products containing TCEP (scenario 3)

In general, TCEP can be a component of e.g. lacquers, paints, glues, adhesives and formulations which are used in e.g. the textile, building and construction and furniture (upholstery) industry. It is to be assumed, that the use of TCEP is restricted to applications which require flame-retarding properties. Industry states, that less than 10 t/y TCEP are applied in paints.

Spraying of paints, lacquers and adhesives is performed in many different industrial and skilled-trade sectors, e.g. vehicle production and repair, treatment and processing of metal and wood and the furniture industry. Lacquers and paints are applied by brushing, rolling, spraying, dipping or covering by pouring. Spraying may be performed manually or automatically. Inhalation exposure caused by droplet aerosols may be a source of exposure. Industry states that lacquers containing TCEP are used in the building industry and textile coating.

In case of mechanical treatment of foams containing TCEP, exposure to TCEP is possible.

In the view of occupational exposure, the application of paints, lacquers, glues, adhesives and flame-retardant formulations are relevant as well as the preparation of paints and cleaning after finishing work. During using formulations the use of PPE (here respiratory protection, gloves and eye protection) is not regarded to be a general measure to reduce exposure.

The concentration of TCEP in end products is assumed to be ≤ 25 %.

Inhalation Exposure

Workplace measurements

Workplace measurement results are not available.

As analogous data for spraying measurement results described in the revised TGD are used (see Appendix IC). Based on measured exposure levels on polyisocyanates, NL derived the following relationship:

Exposure level = 10*fs/30

Fs: percentage of the relevant substance in the paint
30: percentage of polyisocyanate in total paint
10: estimated reasonable worst case exposure level for isocyanates).

Taking a percental content of 25 % TCEP into account, exposure amounts to 8.3 mg/m³. For mechanical or thermal treatment of foams containing TCEP, as a rough estimation, exposure levels are assumed to be below the level assessed for spray applications.

EASE estimations

If no droplet aerosols are produced, inhalation exposure is lower due to the low vapour pressure of TCEP.

Input parameters: T = 20°C, non-dispersive use (vapour pressure < 1 Pa).

Level of exposure: 0 – 0.1 ppm (0 – 1.2 mg/m³)

Conclusions

Inhalation exposure has to be assessed for the use of formulations containing TCEP (e.g. paints, flame-retardant formulations, glues) in fields with lower protection levels, e.g. in small and medium sized companies. The concentration of TCEP is assumed to be ≤ 25 %. For assessing the risks of inhalation exposure during spray application, 8.3 mg/m³ should be taken if spraying techniques are applied (scenario 3a). In case of activities without the formation of droplet aerosols, 1.2 mg/m³ should be taken as representing the reasonable worst case situation (scenario 3b). The duration and frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

Dermal exposure

For the use of formulations containing TCEP in small and medium sized chemical enterprises and the skilled-trade area as well as in enterprises belonging to other industrial areas it cannot be excluded that gloves are not regularly worn and that immediate dermal contacts occur during activities like preparation of paints and flame-retardant coatings, filling and cleaning activities. Taking into consideration that personal protective equipment is not generally worn during painting works, the estimation of dermal exposure levels is performed for the unprotected worker.

Spraying (scenario 3a)

The corresponding exposure for spraying techniques is assessed in application of the EASE model:

Input parameters: T = 20°C, direct handling, wide dispersive use, intermittent

Exposure levels: 1 - 5 mg/cm^2/day.

Considering a TCEP content of ≤ 25 % and an exposed area of 840 cm^2 (hands) an exposure level of 210 - 1050 mg/person/day is obtained.

Dermal exposure during spray painting is due to the deposition of spray mist, back bouncing, contact with contaminated spray gun and possibly also with freshly painted surfaces. The estimates are based on an experimental study in 3 off-shore facilities where containers were painted (Lansink *et al*., 1998) and on studies by HSE and IOM on airless spray application of antifouling paint (HSE, 1999). The Lansink *et al*. (1998) study involved 12 painters, using 3 - 13l of paint with a duration of 4 -21 minutes. A fluorescent tracer was added at 0.0074% (w/w). Exposure levels were presented based on the tracer and a linear extrapolation of exposure related to duration (3 hours, in which 150-200l could have been applied) was done to compare the study with the other studies. The HSE compilation included a total of 70 exposure data provided by 18 separate surveys. The amounts of paint used during spray sessions in the HSE document ranged between 25 and 800l and the spray session ranged from 40 to 360 minutes (median about 180 minutes). On the basis of the 90th percentile of the extrapolated results of Lansink *et al*. (1998) and the 95th percentile of the HSE data a reasonable worst case estimate of 10000 mg on an exposed area of 840 cm² was derived. In consideration of a concentration in formulations of ≤ 25 % dermal exposure through direct skin contact during spraying of the formulations is estimated to < 2500 mg/person/day.

Without the formation of droplet aerosols (scenario 3b)

Input parameters: T = 20°C, direct handling, non dispersive use, intermittent

Exposure levels: 0.1 - 1 mg/cm^2/day.

Considering a TCEP content of ≤ 25 % and an exposed area of 840 cm^2 (hands) an exposure level of 21 - 210 mg/person/day is obtained. The upper level is regarded to represent the reasonable worst case situation.

It cannot be presupposed that eye protection is regularly used. For assessing the risks, hand eye contacts as well as possible splashes to the eye should be considered.

Conclusions

For assessing the risk of daily dermal exposure during painting works and use of glues and adhesives, an exposure level of < 2500 mg/person/day should be taken (analogous data, scenario 3a). For uses without the formation of aerosols, dermal exposure is considerably lower: 210 mg/person/day (scenario 3b).

4.1.1.2.4 Summary

In the EU, the production and import of Tris(2-chlorethyl)phosphate (TCEP) has declined in the past years. In the year 2001, there is no production within the EU, but marketing of TCEP-containing products is still relevant (app.1000 t of TCEP in 2002). The substance is currently produced Poland (amount and use pattern confidential). This is considered in scenario 1.

TCEP is used as a plasticizer with flame-retarding properties in polyurethane, polyester, polyvinyl chloride and other polymers as well as in formulations like paints, lacquers, glues, adhesives and flame-retardant coatings for textiles. The main use of TCEP is the production of unsaturated polyester resins. The use pattern has changed during the last years. One company supplied quantitative information about the application areas of their sales in 2001:

Unsaturated polyester resins	83 %
Acrylic resins	2 %
Adhesives	5 %
Coatings (wood and roofings)	<1 %
Polyurethane	<1 %
Others (not known)	9 %
Cellulose acetate	1 %
Textile	0 %

Due to the low vapour pressure of the substance (< 1 Pa at 20°C), relevant inhalation exposure is likely to occur only if the plastics are handled at elevated temperatures. Industry described, that the substance is neither handled at elevated temperatures nor is used in a powdery state.

For occupational exposure there are 3 main scenarios:

Scenario 1: Production of TECP

Scenario 2: Use of TCEP at ambient temperatures for the production of polymers and formulations

Scenario 3: Use of paints, lacquers, glues, adhesives and flame-retardant coatings.

Relevant inhalation and dermal exposure levels are given in table 4.1.1.6 A and 4.1.1.6 B, respectively. Since no measurement data are available EASE estimates are taken for exposure assessment.

TCEP is a component of different formulations like paints, lacquers, glues and adhesives (scenario 2). For the formulation processes, exposure relevant activities are filling, charging, cleaning, sampling, repair and maintenance activities as well as possibly mixing.

Exposure is to be expected when using paints, lacquers, glues, adhesives and flame-retardant coatings (scenario 3 a - c). The formulations are used in different industrial and skilled-trade areas. Industry states, that less than 10 t/y are applied in paints. During spray application of paints, adhesives and other formulations the formation of droplet aerosols is possible. This is considered in scenario 3a. Scenario 3b represents exposure for activities without the formation of aerosols.If foams containing TCEP are mechanically or thermally treated (e.g.

cutting using hot wires), exposure is assumed to be below the level predicted for spray applications.

Dermal exposure is assessed for the unprotected worker in application of the EASE model or by taking analogous data into account (table 4.2).

Table 4.1.1.6.A Summary of inhalation exposure levels (reasonable worst case) of tris(2-chlorethyl)phosphat which are relevant for occupational risk assessment

	Inhalation exposure							
Exposure scenario	**Form of exposure**	**Activity**	**Duration [hs/day]**	**Frequency [days/year]**	**Shift average [mg/m^3]**	**Method**	**Short-term exposure[mg/m^3]**	**Method**
1. Production	Vapour	drumming, (de)coupling of transfer lines, cleaning, repair	shift length	daily	**1.2** [1)]	EASE	-	-
2. Formulation of paints, lacquers, glues and adhesives (resins), use of TCEP in the production of polymer formulations and products (polyurethane, cellulose acetate).	Vapour	charging, (de)coupling of transfer lines sampling, cleaning, repair, maintenance	shift length	daily	**1.2**	EASE	-	-
4. Use of paints, glues lacquers, adhesives, flame-retardant coatings (25 % TCEP)								
a) spray application	Droplet aerosol	spray application	shift length	daily	**8.3 (3a)**	analogous data	-	-
b) without formation of aerosols	vapour	different techniques, e.g. brushing, rolling	shift length	daily	**1.2 (3b)**	EASE	-	-

LEV: Local Exhaust Ventilation

[1)] The same exposure level is predeicted for workplaces without LEV (due to the low vapour pressure of the substance)

Table 4.1.1.6 B Summary of dermal exposure levels (reasonable worst case) of tris(2-chlorethyl)phosphate which are relevant for occupational risk assessment

Dermal exposure								
Exposure scenario	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Contact level (1)**	**Level of exposure [mg/cm^2/day]**	**Exposed area [cm^2]**	**Shift average [mg/p/day]**	**Method (use of gloves)**
1. Production	Liquid	drumming, (de)coupling of transfer lines, cleaning, repair	daily	intermittent	1	420	**420**	EASE (unsuitable gloves)
2. Formulation of paints, lacquers, glues and adhesives (resins), use of TCEP in the production of polymer formulations and products (polyurethane, cellulose acetate).	Liquid	charging, (de)coupling of transfer lines sampling, cleaning, repair, maintenance	daily	intermittent	1	420	**420**	EASE (irregular use of gloves)
3. Use of resins, paints, glues lacquers, adhesives, flame-retardant coatings (25 % TCEP)								
a) spray application	Droplet aerosol	spray application	daily	intermittent	12 (3a)	840	**< 2500**	analogous data
b) without formation of aerosols	liquid, sticky material	different techniques, e.g. brushing, rolling	daily	intermittent	1 (3b)	840	**210**	EASE

(1) Contact level according to the EASE model

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However, it has to be noted, that the applied model calculations are of preliminary nature and have to be revised as soon as further information becomes available.

4.1.1.3 Consumer exposure

The total scenario of TCEP consumer exposure is depicted in figure 4.1.1.3-1. According to several reports (Pardemann et al., 2000, Sagunski & Roßkamp, 2002, Kersten & Reich, 2003, Becker et al., 2002, Nagorka & Ullrich, 2003, Ingerowski et al., 2001), it is shown, that TCEP will be released from a number of sources which have been treated with flame retardant, namely timber, foam rubber, carpets, plastic materials (e.g. electronic devices, TV, car interior etc.), glues, and lacquers. Upholstery may sometimes be proved with flame retardant. Although there are measurements available of TCEP inside these materials, the degree of migration from the materials is not known.

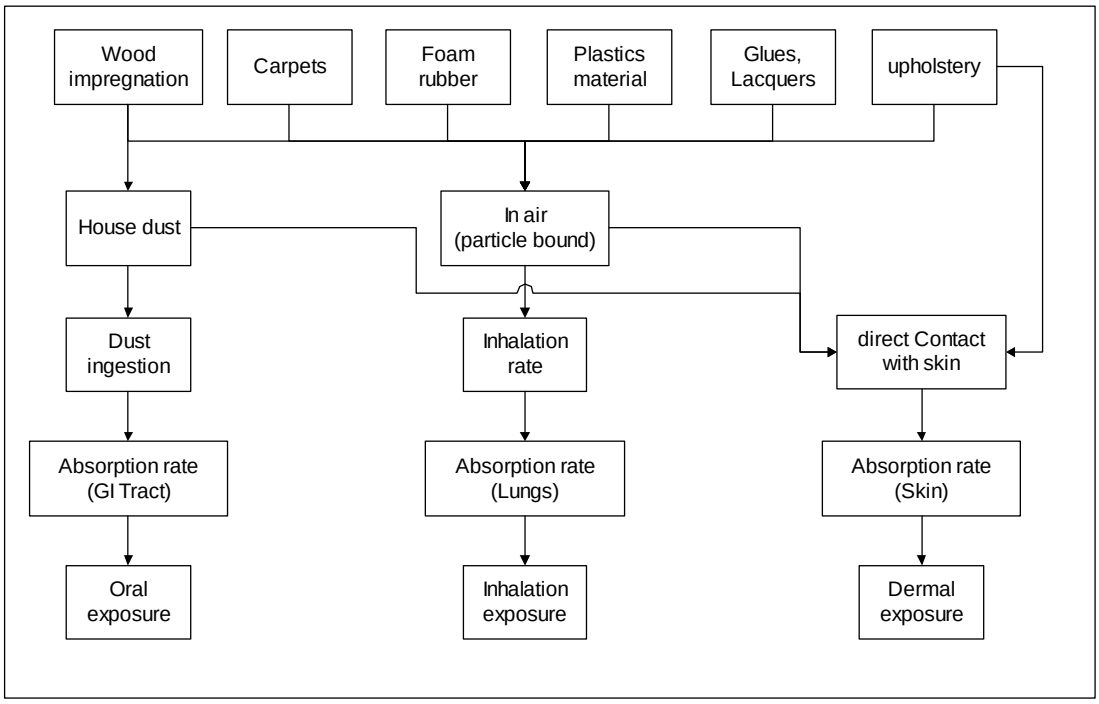


Fig 4.1.1.3-1: Scenario of TCEP exposure for reasonable worst case estimation, according to the above mentioned publications.

TCEP is a non-volatile substance, which does not appear in its gaseous form under normal conditions. Therefore, it is released primarily by abrasion and becomes part of the dust fraction. The latter is divided into two parts, house dust and airborne dust. Dust burden therefore reflects the sum of all the sources (Sagunski & Roßkamp, 2002).

Oral exposure can be referred to dust intake, due to hand-to-mouth behaviour, contamination of articles for daily use, e.g. toys which can be put into the mouth. This pathway of exposure may play a particular role for children and is covered by the hand-to-mouth activities. Inhalation exposure takes place by inhaling airborne particles, and dermal exposure can occur from direct contact with e.g. furniture coverings, as well as with house dust and airborne dust.

Absorption rates in this approach include the desorption of TCEP from dust and the subsequent absorption in the GI-tract or in the lungs and were set to 100% as a worst case approach.

Data on which this assessment is based on

First of all, a study performed by Sagunski et al. (1997) gives an overview on the exposure paths "house dust" and "airborne particles". In this study, house dust was sampled by typical sampling methods taking vacuum cleaners, and the airborne particles were sampled by adsorbing it to polyurethane by drawing air through a sampling advice. This method samples particles as well as possible gaseous TCEP.

House dust:

In an interlaboratory comparative study published by Ingerowski et al. (2001), TCEP dust concentrations have been measured in appr. 1000 German households by three different laboratories using identical methodology (Fig. 4.1.1.3-2). These data and the data from Sagunski (1997) correspond to each other. The range of dust concentration (between zero and 121 µg/kg) is also in agreement with a number of other - smaller - studies (Becker et al. (2002), Bürgi (2002), Hansen et al. (2000), Kersten & Reich (2003), Marklund et al., 2003, Salthammer & Wensing (2002)).

The data published by Ingerowski et al. were taken to evaluate of their distribution for further probabilistic exposure assessment by using the @RISK 4.5 professional tool and MS-EXCEL. The distribution function for TCEP in house dust was best fitted by a log-logistic distribution[8] as shown in Fig. 4.1.1.3-2. The 95th percentile of this distribution is 11.9 mg/kg, and the median 0.6 mg/kg. This distribution covers also the highest values reported by Marklund et al. (2003) in libraries and the other studies mentioned.

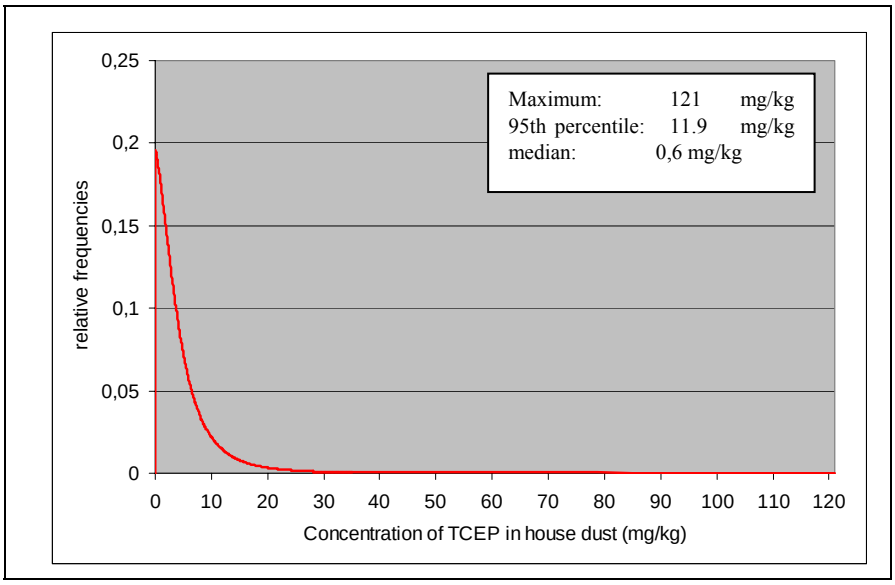


Fig 4.1.1.3-2: Distribution of TCEP concentration results in 983 dust samples taken in German households and analysed in three different labs (Ingerowski, 2001).

It can be assumed that the data give a sound overview about TCEP dust contamination, which can be used for oral exposure assessment by taking commonly accepted dust uptake data.

Airborne dust:

The range of airborne dust concentrations of TCEP as determined in the same study lies between zero and a maximum of 6000 ng/m³, which is in agreement with other authors (Hansen et al., 2000, Bürgi, 2002).

[8] RiskLoglogisticAlt(50%;0,61;90%;8,02; 95%;11,86; RiskTruncate(0;121))

The check for best fit of the data revealed a log-normal distribution[9] with a 95th percentile of 134 ng/m³, and a median of 10 ng/m³. This distribution covers also room air concentrations of max. 30 ng/m³ published by Otake et al. (2001), without specification of dust adsorption, as well as those measured in cars by Wensing et al. (2003).

Toys: From Danish EPA the content of TCEP in a cube for babies was analysed. The cube is intended as a toy for babies (recommended for 0 months and above) to stimulate their senses and their exploring behaviour.This cube is 10 x 10 x 10 cm and weight 100 g. The core of the cube consists of PUR foam. The foam is covered with coloured textile with child motives and at the edges of the cube there are five textile handles. At the handles there are three small plastic rings attached for rattling. A little bell is placed in the middle of the cube imbedded in the PUR foam. About half of the weight of the total cube is estimated to be PUR foam.

Analytical data:
The constituents of the cube were analysed separately. PUR foam had a content of 3300 and 5200 mg TCEP/kg, whereas the textile covering the cube had a content of 160 mg TCEP/kg. A migration test in an aqueous medium has shown that the TCEP is easily dissolved and migrates into the solution.

Exposure assessment:

TCEP exposure was assessed using a probabilistic approach based on the studies mentioned above. Calculations have been performed taking the @RISK-4.5-professional software tool in combination with MS EXCEL.

Oral exposure

Uptake from house dust

TCEP uptake was calculated by the formula

$$E_{TCEP(oral)} = \frac{C_{TCEP,\,dust} * I_{orl,\,dust}}{BW}$$

where $C_{TCEP,\,dust}$ is the dust concentration, $I_{orl,dust}$ is the uptake of dust, and BW is the body weight. According to the age categories of the AUH Report (1995), the oral exposure was estimated for a 1-3 year old child and for a female adult (> 20 years). The dust uptake and body weight[10] data (normal distribution, weighted for 1 to 3 year of age) are taken from the AUH Report (1995). According to these data, the values for this assessment were set as follows: normal dust uptake is set to 20 mg/d and 100 mg/d as upper limit of normal uptake of dust by children. For the adult, the respective values are 2 and 10 mg/d.

[9] @RISK formula: RiskLogNormalAlt(10%;5; 50%;10; 90%;40; RiskTruncate(0;6000))

[10] body weight: RiskNormalAlt(50%;9,1;5%;7,6;RiskTruncate(0;)) (children)
RiskNormalAlt(50%;58;5%;47;RiskTruncate(0;)) (adults)

This estimation of uptake includes soil uptake and therefore leads to a slight overestimate of exposure via dust. It should be mentioned that the upper range of the uptake determined by Calabrese is in agreement with newer data obtained by Freeman & Adgate (2003) who found a daily dust uptake of 100 mg in small children.

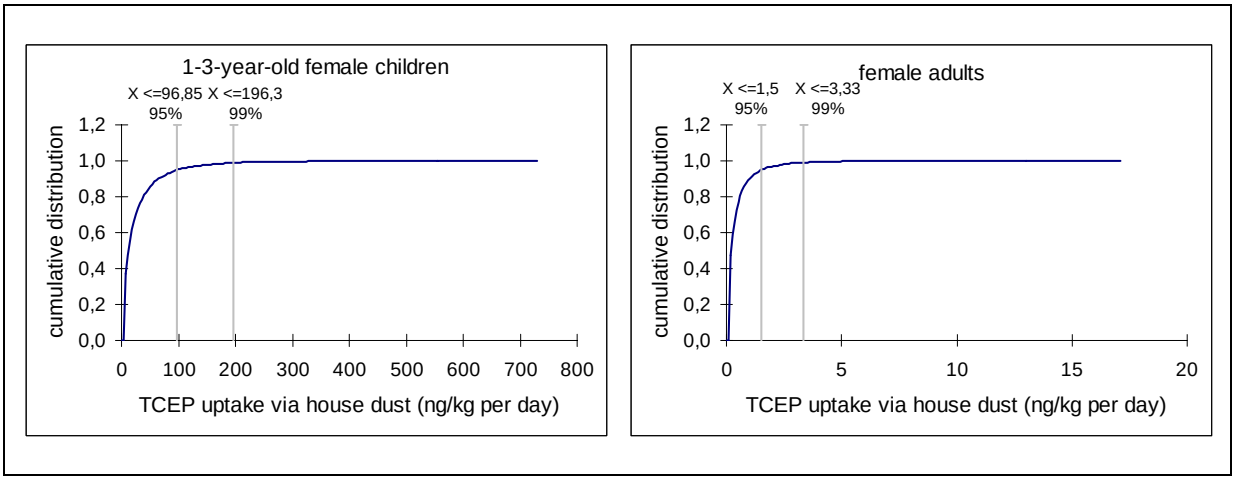


Fig. 4.1.1.3-3: Distributions of oral TCEP uptake from house dust ingestion in three-year-old children and female adults. The results are obtained by taking distribution-based analysis.

As shown in Fig. 4.1.1.3-3, the distribution of TCEP oral uptake is characterised by an extended slope on the right. The 95th and 99th percentile and the maximum are 0.0015, 0.0033 and 0.017 µg/kg per day for female adults. The respective values for children, representing a vulnerable population due to their specific hand-mouth-behaviour are 0.1, 0.2, and 0.7 µg/kg and day, respectively.

Uptake by sucking on toy

It is difficult to estimate the TCEP exposure of children sucking on toys containing TCEP. The rate of migration is affected by factors such as the relative solubility of TCEP in PUR foam and textile and in saliva, the temperature, thickness of textile or foam layer and the physical forces acting on the foam and textile. From a migration test in aqueous media (artificial sweat) it has been found that TCEP easily migrates from the foam cube into the solution. But there are no data available.

Danish calculation (Danish EPA, 2004)

As an exposure scenario it is assumed that a baby of about 3 months plays with a cube during 3 months. Due to mouthing behaviour the cube is sucked intensively and during the 90 days of play about 50% of the total amount of the TCEP -as a worst case scenario- is anticipated to be dissolved and swallowed by the baby. Anticipating an average weight of the baby/ infant of 6 kg during the three month and a total TCEP content of 260 mg (50 g PUR foam with a content of 5200 mg TCEP/kg) a daily dose of 0.24 mg TCEP/ kg bw/d can be calculated (0.5 (total release factor) x 260 mg TCEP / (90 d x 6 kg)).

Discussion

The Danish calculation considers that the TCEP from the foam will migrate through the textile layer during sucking. If this migration is not considered and is only taken the measured TCEP of 8 mg in the textile a daily dose of 7.4 µg TCEP/ kg bw/d can be calculated.

Inhalation exposure

Inhalation exposure to TCEP was calculated by the formula

$$E_{TCEP(inh)} = \frac{C_{air} * Q_{inh}}{BW}$$

where C_{air} is the distribution of the concentration of TCEP in airborne dust, obtained from the Ingerowski et al. study, and Q_{inh} is the respiration volume. The distribution of Q_{inh} was set according to the data given in the AUH report which are based primarily on data published by Finley et al. and in the EPA-Exposure factors handbook.

The evaluation of TCEP uptake by inhalation taking the same approach as described above for dust uptake, revealed a 95th, 99th percentile and a maximum value of 0.07, 0.96, and 3.9 µg/kg per day TCEP for a 3 year old child, and 0.03, 0.4, and 1.4 µg/kg per day for the adult person, respectively. These estimations are in agreement with estimations published elsewhere (Salthammer & Wensing, 2002). Assuming that TCEP will be desorbed totally from the particles and absorbed in the lung alveoles the upper range of this concentration should be taken for characterising a possible risk from inhalation.

Dermal exposure

Textiles (upholstered furniture)

According to measurements which have been performed by Bruckert et al. (1990) an amount of ≈ 130 µg/25 cm² per 24 hours (= 0.217 µg/cm^2/h) of tris(2-chloroethyl)phosphate can be released from upholstery containing an amount of 8 mg/cm².

The area of a person sitting in an armchair covered by textiles accounts for about 1000 cm² (half of both forearms and hands). A four hour contact (e.g. during watching TV) would lead to an amount of ≈ 870 µg/event (= 0.217 µg/cm²/h * 4 h * 1000 cm²). Taking 100 events into account, the yearly total amount of TCEP migrating from an upholstered armchair to the skin accounts for 87000 µg, resulting in an average exposure of ~ 3.9 µg/kg of BW per day, under consideration of a body weight of 60 kg.

For 1-3 year old children, the dermal external contact reveals an amount of 330 µg/event, taking the same conditions as for female adults (0.217 µg/cm²/h * 4 h *380 cm²). Under these conditions, the dermal exposure would account for 10 µg/kg per day (body weight 9.1 kg).

Dermal exposure by airborne dust

The dermal exposure to airborne dust can be neglected due to the very low concentration of 0.6 µg/m³ (the 98th percentile for air concentrations of TCEP), with a contact volume of max. 184 cm³ (calculated by multiplying the total body surface of 18.400 cm^2 (TGD default) with thickness on skin of 0.01 cm (also TGD default). Under these conditions the amount of TCEP in contact with skin is below the nanogram-range.

Dermal exposure by house dust contact

Freeman & Adgate (2003) have estimated a maximum load of ~ 5.0 mg of dust per hand in 1 - 4 year old children. Taking the 98th percentile of TCEP dust concentration reported by Ingerowski et al. (18 ng/mg of house dust), than the total dermal exposure via this pathway would account for ~ 0.18 µg per day (both hands). This value is only applicable for children; the burden resulting from this estimation is 0.018 µg/kg of body weight, considering a child having a bodyweight of 10 kg.

Conclusions

Inhalation:

Taken from the measurements performed by Ingerowski et al. (2001) the 98th percentile for air concentrations of TCEP is 0.6 µg/m³**.** This value lies at the extreme upper range of a huge number of measurements. Although other studies have revealed lower values, due to the high number of measurements, this value is proposed to be taken as a reasonable worst case estimate. For risk characterisation it should be taken into account that the major part is bound to dust and the degree of desorption is unknown.

The model estimate revealed an uptake by inhalation (99th percentiles) of TCEP accounts for 0.4 for adults and 0.96 µg/kg bw and day for children, under consideration of the above mentioned precautions (100% absorption).

Dermal exposure:

The worst case estimation of TCEP by exposure from migration from upholstery accounts for 3.9 µg/kg bw and day. Dermal exposure from airborne dust can be neglected due to the low concentrations of TCEP in dust (max. 0.1 µg/m³). Dermal exposure from house dust accounts for a maximum of 0.02 µg/kg bw per day (in children).

Dermal exposure to TCPE from different sources can be estimated to a total of about ~ 4 µg/kg bw per day. Dermal exposure of children (10 µg/kg bw/day) as related to bodyweight can exceed that in adults.

Oral exposure:

Oral exposure of TCEP is characterised by dust uptake. The estimated amount accounts for 0.0033 µg/kg bw/day for an adult, representing the 99th percentile. Because this pathway may represent a significant source of exposure for children, the 99th percentile representing daily uptake was also estimated for a three year old child and revealed 0.2 µg/kg bw/day.

For babies of about 3 months the significant source could be sucking at a toy. Under worst worst case assumptions values can be achieved up to 240 µg/kg bw per day (Danish calculation).

Total body burdens:

For female adults, a body burden would account for ~ 4.5 µg/kg bw/day, under reasonable worst case conditions, and taking all paths into consideration. The respective value for 1 - 3 year-old children is then 11 µg/kg bw/day. However, for babies of about 3 months a body burden would account up to 240 µg/kg bw per day by sucking on toys, the other paths can be neglected.

4.1.1.4 Indirect exposure via the environment

According to Appendix VII of Chapter 2 of the TGD, the indirect exposure to humans via the environment through food, drinking water and air is estimated for a local and a regional approach. For the local concentrations the default scenario for the formulation of paints is used, representing the local worst case. This scenario is compared to an average intake due to exposure via the regional background concentration. In the Appendix 3, the input data and results of the calculations are presented.

The following input parameters were selected:

annual average local PEC in surface water:	13.59 µg/l
annual average local PEC in air:	0.0038 µg/m³
local PEC in grassland:	8.66 µg/kg
local PEC in porewater of agricultural soil:	18.1 µg/l
local PEC in porewater of grassland:	4.18 µg/l
local PEC in groundwater under agricultural soil:	18.1 µg/l
regional PEC in surface water:	0.0871 µg/l
regional PEC in air:	2.27 x 10^{-4} ng/m³
regional PEC in agricultural soil:	0.061 µg/kg
regional PEC in pore water of agriculture soil:	0.0295 µg/l

The resulting total daily doses are:

$$\mathbf{DOSE_{tot\_local} = 5.842\ \mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}}$$

$$\mathbf{DOSE_{tot\_regional} = 0.0111\ \mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}}$$

The calculated doses comprise the following routes:

Table 4.6

Route	regional model, percentage of total dose	local source model; percentage of total dose
drinking water	22.4	8.85
Air	<0.01	0.01
Stem	66.8	85.9
Root	2.38	2.77
Meat	<0.01	<0.01
Milk	0.02	<0.01
Fish	8.38	2.48

The main route of exposure is the stem for the regional and local approach.

However, it has to be noted, that the applied model calculations are of preliminary nature (i.e. according to TGD "state of the art" methods serving screening purposes) and have to be revised as soon as further information becomes available.

4.1.1.5 (Combined exposure)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

In vivo studies

Oral

Greater than 90% of the orally administered radioactivity (88 mg C14-tris(2-chlorethyl)phosphate (TCEP)kg bw,) in rats were excreted in the urine within 72 hours; 7% via faeces and 1% as carbon dioxide. Elimination from plasma and red blood cells occurred biphasic (half life 3 and 3.4 hours and 1.8 and 10.8 days, respectively). At 0.5 and 4 hours radioactivity was evenly distributed throughout the tissues. Higher concentrations were found in liver, kidney, fat and GI contents (Chadwick et al., 1989).

In an other study in male Wistar rats (5 animals per group) 93% excretion of the orally applied dose (14 mg C14-TCEP/kg bw) in urine, within 168 hours was observed. 6% of the dose was recovered in the feces and 1% in the expired air. The distribution of radioactivity in the tissues was measured 24h after dosing. Lower concentrations of radioactivity were found in brain, muscle, testis, spleen and lung, higher concentrations in liver and kidney. The biliary/fecal ratio was 4.62 at 48 hours. This suggests reabsorption of radioactivity from the gastrointestinal tract. It was supposed that enterohepatic circulation of TCEP occurs (Minegishi et al., 1988).

In a further study on regional brain levels of TCEP male and female F344 rats (3 rats per group) were orally administered 0; 175; 350 or 700 mg/kg bw of the C14-labeled substance as single dosage or 0; 175 and 350 mg/kg bw/d daily within 14 days in females only. The substance was well absorbed from the gastrointestinal tract and distributed in all cerebral regions. Metabolism and excretion nearly totally completed within 72 hours following single or repeated doses. As single dosage (175 mg/kg bw) applied radioactivity was excreted with urine (85%), faeces (less than 10%) and as carbon dioxide. Accumulation of radioactivity was not detected in any cerebral region. After dosage of 350 mg/kg bw acceleration of metabolism and elimination was observed in males whilst in females slightly enhanced plasma concentrations were detected within first minutes post-dosing (Herr et al., 1991).

In a metabolic study male and female B6C3F1 mice and F344 rats were dosed (175 mg/kg bw) with the C14-labeled TCEP either as single dosage or daily within 9 days in rats only (3 mice and 4 rats per group, respectively). In both species excretion within 24 hours was via urine (more than 75%) and faeces (less than 10%). Sex differences in the elimination rate of rats were not detected. Elimination within the first 4 to 8 hours occurred three times faster in mice compared to rats (>70% versus about 40%). Repeated application did not result in a change of metabolism and elimination rate. Metabolites in urine were identical in both species. Main metabolites were bis(2-chloroethyl)carboxymethylphosphate, bis(2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl-2-hydroxyethyl-phosphate glucuronide (Sanders et al., 1990; Burka et al., 1991).

Inhalation

No animal data available.

Dermal

No animal data available.

In vitro studies

In an in vitro study on the metabolism of C14-labeled TCEP in liver slices and liver microsomes by humans and rats liver preparations bis(2-chloroethyl)hydrogen phosphate, 2-chloroethanol were identified as main metabolites. Three further compounds could be found, their structure was not identified. The most important finding of this study is a marked difference in the rate of metabolism and the metabolic pattern between male and female rat microsomes, i.e. male rat liver microsomes metabolized TCEP at a greater rate than did female rat liver microsomes. This difference was not observed in liver slices which gave rise to the assumption of an extramicrosomal metabolism. As in human liver slices and in human

liver microsomes no gender differences in metabolism were observed the finding of gender related differences in rat seems not to have taken into consideration for the risk assessment (Chapman et al., 1991).

4.1.2.1.2 Human data

oral, inhalativ, dermal

No human data available.

4.1.2.1.3 Conclusion

No toxicokinetic data on TCEP have been reported for humans. The substance is well absorbed (> 90% of the dose) and distributed in rats after oral administration. Higher concentrations were found in liver and kidney up to 24h after administration. An enterohepatic circulation is supposed to occur. Elimination from plasma and red blood cells occurred biphasic with a half-life of 3 and 3.4 hours in the beginning and 1.8 and 10.8 days in the second phase. Metabolism and elimination are the same after single and repeated application. Metabolites in urine were identical in rats and mice. Main metabolites were bis(2-chloroethyl) carboxymethylphosphate, bis (2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl) -2-hydroxyethyl-phosphate glucuronide. In the risk characterisation, the rates of oral, dermal, and inhalation absorption are assumed to be 100%.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Oral

Tris(2-chloroethyl)phosphate exhibits moderate acute oral toxicity, with oral LD50 values for female and male rats detected within the range of 430 mg/kg bw (Stauffer Chemical Company, unpublished report 1972) and 1230 mg/kg bw (Ulsamer et al., 1980): The older studies used different lots of a test substance called "Fyrol CEF" of undefined purity. In a study performed according to GLP and to current international guidelines an oral LD50 of 1182 mg/kg body weight resulted for male rats and an oral LD50 of 1123 mg/kg for females. Doses of 800, 1000, and 1260 mg/kg bw were administered orally to groups of 5 male and 5 female rats/dose, using water as vehicle. Deaths (one female at 1000 mg/kg and 4 females and 4 males at 1260 mg/kg) occurred on days 2-4. As clinical signs, pilo-erection and increased salivation were observed in all dose groups; hunced posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities were observed at 1000 and 1260 mg/kg bw only. Survivors recovered within 4 days. Necropsy of the rats that died during the study revealed a very pale cortex in the left kidney of one female dosed at 1260 mg/kg bw; no other macroscopic abnormalities were detected (Huntingdon Research Center, unpublished report 1990).

Inhalation

In a test with rats exposed to an atmosphere saturated with tris(2-chloroethyl)phosphate aerosols (creation of a mist by bubbling air through the sample held at the arbitrary temperature of 170°C, cooling the air, and furnishing it to animals in a small chamber), no rats died after an 8-hours inhalation time (Smyth et al., 1951). In a limit test, five male and five female rats were exposed to a nominal concentration of 25.7 mg/L for 1 hour in a 32 L positive pressure inhalation chamber. The test material was generated by means of a midget impinger. The test animals were observed for 14 days. No mortality resulted, rats revealed moderate lacrymation and salivation, all animals appeared normal within 3 hours (Stauffer Chemical Company, unpublished report 1974).

Dermal

In a limit test using three different lots of undiluted tris(2-chloroethyl)phosphate (no information on purity), four rabbits were each exposed dermally to 2150 mg/kg bw for 24 hours, using occlusive patches. The animals were observed 14 days for mortalities and signs of toxicity. No apparent signs of toxicity were observed. No cholinesterase depression was observed in any of the rabbits 72 hours after treatment (Stauffer Chemical Company, unpublished report 1972).

4.1.2.2.2 Human data

No human data available.

4.1.2.2.3 Conclusion

Tris(2-chloroethyl)phosphate has demonstrated moderate toxicity after oral application, with oral LD50 for rats in the range of 430-1230 mg/kg bw. The inhalation toxicity seems to be low as judged on the basis of test results with rats that survived an 8-hours exposure to saturated substance aerosols or an 1-hour exposure to a nominal concentration of 25.7 mg/L. Acute dermal toxicity in the rabbit is low, the dermal LD50 value was detected to be > 2150 mg/kg bw.

Information on human experience with tris(2-chloroethyl)phosphate is not available. The substance is to be classified as "harmful" according to EEC classification guidelines, labeling with "R 22, Harmful if swallowed" is appropriate.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Animal studies

Abraded and intact skin of 9 albino rabbits was occlusively exposed to 0.5 ml of three lots of unknown purity of the undiluted substance/animal for 24 hours. Results for intact skin: No edema, but erythema grade 1 after 24 hours for all three lots. Results for abraded skin: None of the rabbits demonstrated edema after 24 hours, but all rabbits revealed edema after 72 hours; erythema grad 1-2 were seen in nearly all of the animals at 24 and 72 hours observation. No data on reversibility of these effects and no information on the duration of the study are mentioned (Stauffer Chemical Company, unpublished report 1972).

The skin of three albino rabbits was semi-occlusively exposed to 0.5 ml of the undiluted substance (purity > 99%) per animal for 4 hours in a Draize test performed according to GLP and to international test guidelines (EECB.4/OECD 404). Result: All rabbits revealed slight erythema that reversed within 24 hours (Huntingdon Research Center, unpublished report 1991; Hoechst AG, unpublished report 1988a). In a second test according to test guidelines EECB.4/OECD 404 the skin of 3 albino rabbits was semi-occlusively exposed to 0.5 ml of the undiluted substance (purity 99.5%) per animal for 4 hours. Result: 1/3 rabbits revealed slight erythema grade 1 that reversed within 24 hours (Hoechst AG, unpublished report 1988a).

Human data

No human data are available.

4.1.2.3.2 Eye

Studies in animals

In a Draize test according to GLP and to international test guidelines (EECB.5) instillation of 0.1 ml of undiluted tris(2-chloroethyl)phosphate (purity > 99%) into the eyes of each of three rabbits resulted in mild conjunctival irritation (edema grade 1 in all animals on day 1, conjunctival redness grade 1 in all animals for a period of 1 or 2 days). Three days after instillation all effects had reversed. No corneal damage or iridial inflammation was seen (Huntingdon Research Center, unpublished report 1991b). Similar results demonstrated a second study according to GLP and to international guidelines (EECB.5/OECD TG 405): The installation of 0.1 ml of the undiluted substance (purity 99.5%) resulted in weak conjunctival irritation (oedema grade 1 in 2/3 animals on day 1, conjunctival redness grade 2 in all rabbits on day 1). Twenty four hours after instillation all effects had reversed (Hoechst AG, unpublished report 1988b). In a study performed in 1972 with three different lots of unknown purity, no local effects on rabbit eyes were detected (Stauffer Chemical Company, unpublished report 1972).

Human data

No human data available.

4.1.2.3.3 Conclusion

Human data on local irritant substance properties are not available. Tris(2-chloroethyl)phosphate exhibits only weak local irritation to skin and to the conjunctivae of the eyes of rabbits. Thus, TCEP is not considered to be a skin and eye irritant.

4.1.2.4 Corrosivity

Animal studies

From the data presented in the preceding text it is evident that tris(2-chloroethyl)phosphate is not a corrosive substance.

Human data

No human data available.

Conclusion

Tris(2-chloroethyl)phosphate is not a corrosive substance, as judged on the basis of animal studies presented in the preceding text.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

The skin sensitisation potential of TCEP was evaluated with the Buehler method (Mobil (1983) as cited by BG Chemie, 1995). Guinea pigs (5 animals each per sex) were induced once per week with the undiluted substance over three weeks. Two weeks after the 3rd induction the animals were challenged with the undiluted substance. No allergic reactions were seen (oberservation time 24 or 48 h). No further data are available.

4.1.2.5.2 Human data

No human data available.

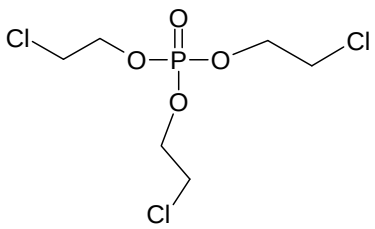
4.1.2.5.3 Data available on structurally related chloroalkyl phosphates

Tris(2-chloroethyl) phosphate shows structural analogy to the two priority substances Tris(2-chloro-1-methylethyl) phosphate and Tris[2-chloro-1-(chloromethyl)ethyl] phosphate being assessed by Ireland/UK (cf. EU RAR TCPP (2006), EU RAR TDCP (2006). Therefore, information about these chloroalkyl phosphate esters is included here and will be used for a read-across approach to assess the skin sensitisation potential of TCEP.

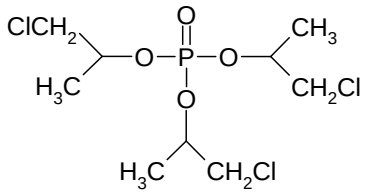
4.1.2.5.3.1 Chemical structures

The substance structures are shown below.

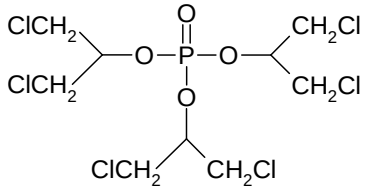
Tris(2-chloroethyl) phosphate (TCEP)



Tris(2-chloro-1-methylethyl) phosphate (TCPP)



Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP)



4.1.2.5.3.2 Physico-chemical and alkylating properties

Selected physico-chemical properties of the three chloroalkyl phosphates used for the read-across are listed in table 4.7. The molecular weights and logPow values are in the same range, whereas solubility decreases in the order from TCEP to TDCP.

Table 4.7

Selected physico-chemical properties of TCEP, TCPP and TDCP

	TCEP	TCPP	TDCP
Molecular weight (g/mol)	285	327	430
Water solubility (mg/l, 20°C)	7820	1080	18.1
Partition coefficient (LogPow)	1.78	2.68	3.69

The alkylating properties of chloroalkyl phoshates have been investigated by several authors, e.g. Crook and Haggis (1969), Bissell (1977), Levchik et al. (2005). The results show that TCEP, TCPP and TDCP tend to N-alkylate aromatic amines and the urethane structure of PU foams at higher temperatures (> 140°C) under special reaction conditions. But it is unlikely that such reactions will occur with primary aliphatic amines at lower temperatures in aqueous media (Belke et al., 2003). Thus, it can be concluded that N-alkylation reactions at the nitrogen atoms of the polypeptide backbone or of ε-amino groups of proteins will not occur at body temperature in physiological media. Therefore, the potential of TCEP and analogues to be bound covalently to proteins via alkylation can be regarded as negligible.

4.1.2.5.3.3 Animal data

Tris(2-chloro-1-methylethyl)phosphate (TCPP)

No evidence of skin sensitisation was found in a 1979 study (SafePharm, 1979). Following a range-finding test, 0.1 mls of a 5% solution of TCPP was selected for intradermal induction followed after 24 hours, by application of undiluted test substance for 48 hours, as the topical induction. 10% sodium lauryl sulphate was applied 24 hours prior to the topical induction. 10 guinea pigs were treated with TCPP and 4 remained untreated as controls. Two weeks after topical induction, the neat test substance was applied for 24 hours under occlusive dressing. There was no significant response after challenge. While this study is not GLP compliant (performed in 1979), its result is considered to be acceptable as a negative result.

In a GLP-compliant Local Lymph Node Assay (LLNA) conducted in accordance with OECD Guideline No. 429 TCPP was considered to be a non-sensitiser (EU RAR TCPP, 2006). Groups of four CBA/Ca mice were treated with 25 µl undiluted TCPP or concentrations of 50% or 25% v/v in acetone:olive oil 4:1 for three days. A further group of four mice received the vehicle alone. Five days following the first topical application, all mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing a total of 20 µCi ^{3}H-methyl thymidine (specific activity 2.0 Ci/mmol). All mice were terminated five hours after injection. Stimulation indices of 1.55, 1.97 and 1.56 were recorded for concentrations of 25, 50 and 100% (v/v), respectively.

In two personal communications from occupational physicians from two EU TCPP manufacturing sites, the Irish rapporteur was informed that there has been no evidence of skin sensitisation among workers.

Also, in a personal communication from the regulatory affairs manager of a US-based TCPP manufacturing site, the Irish rapporteur was informed that worker medical records at the site for 13 years preceeding 2001 show no evidence of health effects, including skin sensitisation, associated with exposure to and handling of TCPP.

In conclusion, evidence from a guinea pig study as well as from a local lymph node assay indicates that TCPP does not possess significant skin sensitisation potential. No information is available on the respiratory sensitisation potential of TCPP.

Tris[2-chloro-1-(chloromethyl)ethyl]phosphate (TDCP)

In a well-reported guinea pig maximisation test conducted to OECD guidelines, TDCP showed no evidence of dermal sensitisation (Manciaux, 2001, EU RAR TDCP, 2006). A group of 20 test animals received an intradermal injection of 25% TDCP in corn oil and a topical application of 100% TDCP, preceded by a topical application of 10% sodium lauryl sulphate (administered on induction day 7). 10 control animals received vehicle only. Subsequent dermal challenge with 100% TDCP resulted in no signs of erythema or oedema in any of the test or control animals. A positive control study was included using mercaptobenzothiazole, which gave appropriate responses.

In conclusion, evidence from a study in guinea pigs indicates that TDCP does not possess significant skin sensitisation potential. No information is available on the respiratory sensitisation potential of TDCP.

4.1.2.5.4 Conclusion

Human data on sensitizing properties of tris(2-chloroethyl)phosphate are not available. An animal skin sensitisation study (Buehler Test) showed no skin sensitizing potential of TCEP. No further testing is recommended based on read across from sensitisation data of the two other chloroalkyl phosphate esters TCPP and TDCP (substances of the 4th EU Priority list) which represent structurally related substances and have been tested in guinea pigs and in a local lymph node assay. The results indicate that these substances do not possess significant skin sensitisation potential.

Based on all information on the three structurally related chloroalkyl phosphates (results of animal testing, similarity in physicochemical data and chemical structures, as well as alkylating properties of TCEP, TCPP and TDCP) it is concluded that TCEP should be non-sensitizing to humans.

No information is available on the respiratory sensitisation potential of TCEP and the other two chloroalkyl phosphates.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Animal data

The most reliable repeated dose toxicity studies used the oral route (gavage and feeding) of exposure to tris(2-chloroethyl)phosphate. Results of these studies give the indication that brain and kidney are the main target organs of toxicity, although there is a species and sex-related variability. There is also clear evidence that mice were less sensitive to the effects of tris(2-chloroethyl)phosphate than rats. Results of studies with repeated application of tris(2-chloroethyl)phosphate are summarized in Table 4.11 Several experimental investigations are long-term/long-life studies designed for examination of carcinogenicity. Therefore, for long-term studies see also 4.1.2.8.

4.1.2.6.1.1 General endpoints

- ***Oral***

Gavage studies (rat and mouse)

In order to evaluate toxicological effects occurring as a result of repeated daily dosing with tris(2-chloroethyl)phosphate for expected lifespan, and to determine the concentrations for subsequent carcinogenicity trials 14-day and 16-week studies in rats and mice were performed. Since many organophosphorous chemicals demonstrate neurotoxic activity by inhibiting the enzyme cholinesterase, the ability of tris(2-chloroethyl)phosphate to inhibit this enzyme was also studied (Matthews 1990, NTP 1991). In these studies tris(2-chloroethyl)phosphate was administered once per day, 5 days/week by gavage in corn oil.

14-day studies

<u>Rats</u>

Groups of 5 male and 5 female F344/N rats (8-9 weeks old) received tris(2-chloroethyl)phosphate (purity 98%) in doses of 0, 22, 44, 88, 175, and 350 mg/kg bw/d for 12 doses over 16 days. No mortality due to tris(2-chloroethyl)phosphate, no changes of body weight gain and no clinical signs of toxicity and/or neurotoxicity were observed in both sexes up to 350 mg/kg bw/d. At 175 and 350 mg/kg bw/d the mean absolute and relative kidney weights of male rats were 10 and 12% higher than those of the controls, respectively. Liver weights of females in the 350 mg/kg bw/d dose group were significantly (17%) higher than those of the controls. In addition a significant decrease of absolute and relative lung weights in female rats receiving 88 to 350 mg/kg bw/d ($p \leq 0.05$) were seen. Nevertheless all organ weight changes were observed without further corroborating findings, therefore these findings were not considered to be of toxicological significance. No other organ weight variations attributable to tris(2-chloroethyl)phosphate were observed in rats of both sexes. Cholinesterase activity determined on fresh sera collected at necropsy was not reduced in dosed male rats, but was decreased by 18% and 20% in females at 175 ($p \leq 0.01$) and 350 mg/kg bw/d ($p \leq 0.05$) tris(2-chloroethyl)phosphate. This finding was only considered as a biomarker of exposure, but not considered as toxicologically relevant because statistically significant inhibition of plasma ChE of ≥20% is regarded as toxicologically adverse. (Based on the published policies on cholinesterase inhibition from WHO/UNEP (1990), U.S.EPA (Sette, 1997; Dorsey, 1997) and JMPR (Reprt, 1998) a ststistical significant AchE inhibition of ≥ 20% is regarded as toxicologically relevant ("adverse"). . No gross or histopathologic lesions were observed at any dose level.

The NOAEL for male and for female rats was 350 mg/kg bw/d (NTP 1991, Matthews 1990).

Mice

Groups of five B6C3F1 mice (9-10 weeks old) per sex were treated by gavage with 0, 44, 88, 175, 350, and 700 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) for 12 doses over 16 days. There were no treatment-related effects on mortality, body weight gain, absolute and relative organ weight or histopathological abnormalities up to 700 mg/kg bw/d. Gavage trauma accounted for the deaths of two males (each one at 175 mg/kg bw/d and 350 mg/kg bw/d) and of one female at 700 mg/kg bw/d. Mice of both sexes given 350 and 700 mg/kg bw/d tris(2-chloroethyl)phosphate exhibited ataxia and convulsive movements during the first 3 days of dosing. These symptoms did not persist after the first three days (no more data). In treated mice of both sexes serum cholinesterase activity was similar to that of controls.

The overall NOAEL for male and female mice for convulsion and ataxia was 175 mg/kg bw/d (NTP 1991; Matthews, 1990).

16-week studies

During week 4, the two highest doses were incorrectly prepared and administered for the first three days of the week. Rats and mice of these two highest dose levels received a double dose during these three days in week 4.

Rats

Groups of 10 male and 10 female F344/N rats received tris(2-chloroethyl)phosphate (purity 98%) in doses of 0, 22, 44, 88, 175, and 350 mg/kg bw/d for 16 weeks (female) or 18 weeks (male). During the course of the subchronic toxicity study (4th week) two females each died

at 350 and 175 mg/kg bw as a result of the reported error in test substance preparation. Female rats receiving overdoses exhibited signs of toxicity, including ataxia, excessive salivation, gasping, and convulsion. The overdosed male rats showed no signs of toxicity, and none died. During the 16-week study, there were additional deaths of rats, one male receiving 175 mg/kg bw/d, 5 males receiving 350 mg/kg bw/d, and three females receiving 350 mg/kg bw/d that were not associated with the overdosing. The deaths of one male rat in the 350 mg/kg bw/d group and one male and two female rats in the 22 mg/kg bw/d dose group occurred as a result of gavage trauma. Female rats receiving 175 and 350 mg/kg bw/d experienced occasional periods of hyperactivity after dosing. Periodic convulsions were noted in 350 mg/kg bw/d dosed females but not in males during week 12. Final body weights of female rats receiving 350 mg/kg bw/d were 20% greater than those of controls; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar. The relative liver and kidney weights were significantly ($p \leq 0.01$) increased in males receiving 350 mg/kg bw/d and in females receiving 44 to 350 mg/kg bw/d. In males given 350 mg/kg bw/d the increases in relative liver and kidney weights were 22% and 26%, respectively. In females the increases in relative liver weights were 13%, 13%, 19%, and 50% in the 44, 88, 175, and 350 mg/kg bw/d groups, respectively; the increases in relative kidney weight were 8%, 11%, 11% and 22% in the 44, 88, 175 and 350 mg/kg bw/d groups, respectively. These effects occurred in the absence of any apparent histopathological lesions in either of these tissues. At 350 mg/kg bw/d, female rats showed decreases in absolute brain (11%) and thymus (19%) weights. Microscopy revealed significantly increased lesions in the brains of male and female rats. Tris(2-chloroethyl)phosphate-related neuronal necrosis occurred in the hippocampus and thalamus of female rats at 175 and 350 mg/kg bw/d and, to a lesser extent, in male rats at 350 mg/kg bw/d (mild damage, not significant). These effects were more pronounced in female rats than in males. Loss of neurons in the area of the hippocampus was observed in 10/10 females and 2/10 males of the 350 mg/kg bw/d group, and in 8/10 females of the 175 mg/kg bw/d dose group. There was a significant sex-related difference in dose-response ($p=0.001$). The hippocampal damage was significant in female rats receiving 350 and 175 mg/kg bw/d, and their severity increased with dose in female rats ($p=0.0001$). The affected neurons were noted predominantly in the dorsomedial portion of the pyramidal row of the hippocampus. Necrosis and loss of thalamic nuclei were also observed in two female rats receiving 350 mg/kg bw/d. In some cases, there was tissue mineralization and microgliosis associated with loss of pyramidal cells in the hippocampus. Cholinesterase activity determined in serum at necropsy was 75% and 59% of the control value in female rats receiving 175 or 350 mg/kg bw/d tris(2-chloroethyl)phosphate, respectively ($p \leq 0.01$), but was not reduced in male rats.

Overall, significant effects on organ weights were seen from increased relative liver and kidney weights of males receiving 350 mg/kg bw/d and in females receiving 44 to 350 mg/kg bw/d. Since the increases in relative liver and kidney weights in males and in females could not be correlated to histopathologic effects, these findings in organ weights were not considered to be toxicologically adverse in this study. The most relevant toxic effects observed in this study were mortality in rats receiving 350 mg/kg bw/d and brain lesions seen in the hippocampal region in rats receiving 175 or 350 mg/kg bw/d. The NOAEL for neuronal effects in the hippocampus in female rats was 88 mg/kg bw/d and in male rats 175 mg/kg bw/d, respectively (NTP 1991, Matthews 1990).

Mice

Groups of 10 B6C3F1 mice of each sex were treated with 0, 44, 88, 175, 350, and 700 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) for 16 weeks. There were no treatment-related deaths, although gavage trauma caused the deaths of three males (each at 175, 350, and 700 mg/kg bw/d) and of two females (each at 175, and 350 mg/kg bw/d) before the end of the study. No differences in body weight gain and final mean body weight, or differences in cholinesterase activity were noted in mice receiving 44 to 700 mg/kg bw/d tris(2-chloroethyl)phosphate. The mean absolute liver weights were significantly ($p \leq 0.01$) increased in females receiving 175 to 700 mg/kg bw/d (14%, 20%, and 13%, respectively) and in male mice receiving 700 mg/kg bw/d (5%). The liver-to-body weight ratios, however, were not increased. The increased absolute liver weights were seen without corroborative findings in morphology. In male mice in the 175 to 700 mg/kg bw/d dose groups the absolute kidney weights were significantly ($p \leq 0.01$) decreased (5%, 10%, and 20%, respectively). The kidney-to-body weight ratios, however, were not affected. Histopathologically examinations of the kidneys showed epithelial cells with enlarged nuclei (mild cytomegaly and karyomegaly) in all animals of both sexes given 700 mg/kg bw/d. Lesions were observed primarily in the proximal convoluted tubules of the inner cortex and outer strip of the outer medulla and to a lesser extent in the straight portion of the loops of Henle in the medulla. The hippocampal and thalamal lesion like in rats was not observed in either sex of mice. Sperm count in mice was slightly decreased ($p = 0.05$) in animals receiving 700 mg/kg bw compared to that of controls.

Organ weight determinations revealed statistically significant increased livers in females given 175 to 700 mg/kg bw/d and in males given 700 mg/kg bw/d without further corroborating findings, so these findings were not considered to be toxicologically adverse. Relevant toxic effects were present in male and female mice receiving 700 mg/kg bw/d. There were renal lesions (mild cytomegaly and karyomegaly) and significantly reduced mean absolute kidney weights.

A NOAEL for renal effects for mice of both sexes was estimated at 350 mg/kg bw/d (NTP 1991, Matthews 1990).

Adverse effects on the brain in rats and mice after repeated exposure to tris(2-chloroethyl)phosphate will be discussed under chapter 4.1.2.6.1.2 Specific endpoints - neurotoxicity

103-week studies

Information on neoplastic findings of tris(2-chloroethyl)phosphate in experimental animals obtained from these carcinogenicity studies (NTP 1991, Matthews 1993) are described in detail in section 4.1.2.8.

Rats

Groups of 60 F344/N rats per sex received 0, 44, or 88 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) in corn oil by gavage, once per day, 5 days per week, for up to 103 weeks. An interim evaluation (necropsy, histopathology, hematology, and clinical chemistry) was performed on ten animals per sex per group after a treatment period of 66 weeks. At 66 weeks, one female receiving 88 mg/kg bw/d dies on day 261, and one vehicle control male died on day 408; the remaining lived until interim sacrifice on day 458 and 459. There were no adverse effects seen on body weight gain or hematology. There were

significantly decreases in serum alkaline phosphatase and alanine aminotransferase in females receiving 88 mg/kg bw/d ($p \leq 0.01$). Slight increases in mean absolute liver and kidney weights were recorded in males at the same dose level; for relative liver and kidney weights statistically significant increases of 14% and 20%, respectively, were observed ($p \leq 0.01$). At this time point there were renal tubule adenomas in one male receiving 88 mg/kg bw/d. Focal brain lesions were located in the cerebrum and thalamus. In cerebrum and thalamus, local necrosis and accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia were observed in 3/10 females given 88 mg/kg bw/d.

At 103 weeks the final mean body weights of test and control groups were similar. There were no clinical signs of toxicity. Reduced survival was noted in male and female rats at 88 mg/kg bw/d (males: 51% survival compared to 78% in controls; females 37% compared to 66% in controls $p \leq 0.01$). Female rats dying early or sacrificed while moribund frequently had brain lesions, by contrast male rats did not. The mean treatment-related effects of tris(2-chloroethyl)phosphate occurred in the kidney and the brain. A significant increase of focal hyperplasia of the renal tubule epithelium was recorded in both treated male and female rats (in males: 0/50, 2/50; and 24/50 and in females: 0/50, 3/50, and 16/50). Hyperplasia of the renal tubule epithelium occurred in the convoluted tubules of the cortex and were characterized by stratification of the epithelial cells with partial to complete obliteration of the tubule lumens. A summary of the incidence of hyperplasia in the renal tubules in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.8

Table 4.8 Incidence of hyperplasia in the renal tubules of F344/N rats in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**44 mg/kg bw/d**	**88 mg/kg bw/d**
male (number examined)	50	50	50
Hyperplasia, focal		1 (2%)	1(2%)
Mineralization		1 (2%)	
Epithelium, hyperplasia		1 (2%)	1 (2%)
Epithelium, hyperplasia, focal			18 (36%)
Epithelium, hyperplasia, multifocal			4 (8%)
female (number examined)	50	50	50
Epithelium, hyperplasia			4 (8%)
Epithelium, hyperplasia, focal		3 (6%)	11 (22%)
Epithelium, hyperplasia, multifocal			1 (2%)

For this relevant toxic effect there was a clear dose-response relationship in the kidneys in male and female rats at both treatment groups, 44 and 88 mg/kg bw/d, thus, a NOAEL for kidney lesions could not be estimated in this study. So, 44 mg/kg bw/d is considered to be the LOAEL for kidney lesions in animals of both sexes.

A marked increase in the incidence of degenerative lesions of the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia) was noted in over 44% of female rats receiving 88 mg/kg bw/d. In contrast to the findings in the 16-week studies, degenerative lesions were widely distributed in the gray and white matter of the brain stem and cerebral cortex of high-dose females and, to a lesser extent in male rats. Degenerative lesions consisting of gliosis, mineralization, hemorrhage, and/or hemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate; whereas similar lesions were seen in only a few dosed males, however, they were assessed as toxicologically relevant, although these findings were not significantly increased in incidence and severity, nevertheless they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Accordingly, the NOAEL for brain lesions is 44 mg/kg bw/d for male and female F344/N rats (NTP 1991, Matthews 1993). A summary of non-neoplastic brain lesions in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the special part: “Other special studies – Neurotoxicity”, where more details can be found.

Mice

Groups of 60 B6C3F1 mice of each sex received 0, 175, or 350 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) by oral gavage on the same dosing schedule. Ten animals per sex per group species were predetermined for interim evaluation (necropsy, histopathology, hematology, and clinical chemistry) after 66 weeks. At 66 weeks, there were no effects seen on mortality, body weight gain, hematology or clinical chemistry. Microscopy of the kidneys revealed hyperplasia of tubule epithelial cells in two males receiving 350 mg/kg bw/d.

At 103 weeks mortality and body weight gain were similar among dosed groups of male and female mice and their respective controls. The main target organ for toxicity was the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in tris(2-chloroethyl)phosphate treated male and female mice given 175 or 350 mg/kg bw/d (16/50, and 39/50 males; and 5/49 and 44/50 females). This finding was present in approximately 80% of mice receiving 350 mg/kg bw/d and less frequently in mice receiving 175 mg/kg bw/d, while no signs of cellular necrosis were reported from both dose levels. The affected cells were in the proximal convoluted tubules of the inner cortex and outer stripe of the outer medulla and, to a lesser extent, in the pars recta of the loops of Henle in the outer medulla. The lesion was minimal in most mice and consisted of only a few widely scattered tubule epithelial cells with enlarged hyperchromatic single nuclei. In concurrent controls, karyomegaly were observed in 2/50 males and in 0/50 females. An overview of selected renal tubule cell lesions in B6C3F1 mice in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.9.

Table 4.9 Selected renal tubule cell lesions in B6C3F1mice in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**175 mg/kg bw/d**	**350 mg/kg bw/d**
male (number examined)	50	50	50
Karyomegaly	2	16	39**
Hyperplasia (original + step sections)	1	0	3
female (number examined)	50	49	50
Karyomegaly	0	5*	44**
Hyperplasia (original + step sections)	0	1	2

* significantly different (p≤0.05); ** significantly different (p≤0.01) from the control group by logistic regression tests

There was a clear dose-response relationship for this relevant toxic effect in the kidneys in male and female mice at both treatment groups. The NOAEL for kidney lesions for mice could not be estimated in this study. Therefore, 175 mg/kg bw/d is considered to be the LOAEL for changes in kidney morphology in B6C3F1 mice of both sexes. In the liver, there was an increased incidence of foci of cytologic alteration, particularly eosinophilic foci in males (control: 0/50, 0%; 175 mg/kg bw/d: 3/50, 6%; 350 mg/kg bw/d: 8/50, 16%), although the incidence of basophilic (control: 1/50, 2%; 175 mg/kg bw/d: 2/50, 4%; 350 mg/kg bw/d: 1/50, 2%) or clear cell foci (control: 4/50, 8%; 175 mg/kg bw/d: 1/50, 2%; 350 mg/kg bw/d: 5/50, 10%) were not. Eosinophilic, basophilic, and clear cell foci comprise a morphological continuum with hepatocellular adenoma and are believed to be precursors of hepatocellular neoplasms. In female mice there was no increase in the incidence of hepatocellular foci. An overall NOAEL for non-neoplastic lesions for male and female mice could not be determined (NTP 1991, Matthews 1993).

- *Diet studies (rat and mouse)*

28-day study

Rats

In a 28-day dietary range-finding study (Stauffer Chemical Company, unpublished report 1980a), groups of 10 male and 10 female Sprague-Dawley CD rats were administered at dose levels of 0, 500, 850, 1500, and 2000 ppm (equivalent to 0, 42, 72, 125, and 163 mg/kg bw/d in males, and 0, 50, 88, 144, and 191 mg/kg bw/d in females, calculation on nominal dietary levels) of tris(2-chloroethyl)phosphate in the diet. The mean weekly test substance consumption values reported in the study report were calculated to mean values for the period

of 12 weeks. Additionally two weeks after initiation of dosing, the 200 ppm dose level (equivalent to 19 mg/kg bw/d in males, and 20 mg/kg bw/d in females) was increased to 4000 ppm (equivalent to 293 mg/kg bw/d in males and 334 mg/kg bw/d in females), and in another dose group receiving 350 ppm (equivalent to 30 mg/kg bw/d in males and 38 mg/kg bw/d in females) for three weeks the dose level was increased to 8000 ppm (equivalent to 495 mg/kg bw/d in males and 508 mg/kg bw/d in females) for one week. This study was not conducted in line with the requirements of the guideline testing protocols of B.7/ OECD TG 407; it differs in some respects from the published guidelines; following significant deficiencies exist: only a small selection of parameters in clinical biochemistry; no organ weight assessment, no histopathology. However, basic data are given in this range finding study, and they were used as supporting information. There were no test substance-related deaths. The mean body weights of treated rats at all dose levels were comparable to the control rats. A statistically significant decrease in food consumption after one week of treatment was observed in males and females at 8000 ppm (equivalent to 495 mg/kg bw/d in males and 508 mg/kg bw/d in females, $p \leq 0.05$). Hematology and clinical chemistry examinations showed no biologically significant treatment-related alterations. The seminal vesicles and/or prostate were smaller than normal in one rat in the dose group of 1500 ppm (equivalent to 125 mg/kg bw/d) and in three males receiving 8000 ppm (equivalent to 495 mg/kg bw/d). The testes of one male were dissimilar in size and weight, which may have been related to ingestion of the test substance.

The results of this study provided the basis for selection of appropriate dose levels for the following 3-month study.

3-month study

Rats

In a three month dietary toxicity study mostly according to OECD test guideline 408 (no microscopy of the brain) five groups of Sprague-Dawley CD rats (20/sex/group) were fed 0, 400, 1000, 3000, and 8000 ppm (equivalent to 0, 26, 65, 192, and 506 mg/kg bw/d in males; and 0, 30, 75, 215, and 586 mg/kg bw/d in females, calculation of intake based on nominal concentrations) tris(2-chloroethyl)phosphate (purity commercial grade) in a commercial diet (Stauffer Chemical Company, unpublished report 1980b).

No treatment-related deaths and clinical signs were seen in rats of both sexes. Body weights and the mean weekly food consumption values of males and females consuming 8000 ppm tris(2-chloroethyl)phosphate were significant ($p \leq 0.05$) lower (13-16%; 11-18% respectively) than controls. Food consumption was also depressed in females of the 3000 ppm dose group, however, the difference was only 5% and may be biologically significant. Final body weights were lower in males and females at 3000 ppm (-7% and -8%, resp.) and at 8000 ppm (-18% and -17%, resp.). Analysis of clinical chemistry, hematology, urinalysis, and cholinesterase revealed no compound-related effects. The mean relative liver and kidney weights were significantly increased in both sexes of 3000 ppm and 8000 ppm (equivalent to 506.42 mg/kg bw/d in males, and 586.22 mg/kg bw/d in females, $p \leq 0.05$) dose groups. In male rats mean relative liver weight was 19% higher at 3000 ppm and 22% at 8000 ppm when compared to the concurrent controls. In females increases of 9% and 30% were noted, respectively. For mean relative kidney weights the following significant increases were determined: in males: 9% at 1000 ppm, 15% at 3000 ppm and 22% at 8000 ppm; and in females: 12% at 3000 ppm and 13% at 8000 ppm, respectively. There were a number of tubular hyperplasias in control and treatment groups with a tendency to increase in incidence at higher dosages (total

incidences 6/20, 4/20, 7/20, 8/20 and 11/20 at 0, 400, 1000, 3000 and 8000 ppm). The attribution of the small raise in numbers at 1000 and 3000 ppm compared to the control level to the treatment may be questionable, but the incidence at 8000 ppm was markedly above that of the control group. The mean severity seems to be elevated at 400 ppm compared to the control level, but no dose-dependent increase in severity was seen at higher dose levels (mean severity: 1.3, 2.75, 1.1, 1.0 and 1.2 at 0, 400, 1000, 3000 and 8000 ppm). In the light of the mean severity scores at higher dosages, the two males out of 4 affected at 400 ppm, which showed marked or severe regenerative hyperplasia should be considered as incidental. There were no other gross or histological lesions attributed to tris(2-chloroethyl)phosphate treatment. The relative mean weights of the gonads and the brain were also reduced in dose groups at 3000 and/or 8000 ppm. Except for absolute heart effects that were lower in high dose males and females, the absolute mean weights of other organs were similar to those of the controls. This and the absence of other treatment-related gross or histological lesions support that the relative increases in organ weights might be due to the lower body weight gain at 3000 and 8000 ppm. This reduction in growth could be attributed to lower food consumption. The increase in incidence in regnerative hyperplasia in the renal cortex at the 8000 ppm is considered to be related to the treatment. Therefore 3000 ppm is the NOAEL (equivalent to 192 mg/kg bw/d in males; and 215 mg/kg bw in females).

Little information was obtained from an early diet study in rats (Stauffer Chemical Company, unpublished report 1975, cited as summary without detailed information in Ulsamer et al. 1980). Groups of 10 male and 10 female rats (strain not specified) were administered up to 0.5% (250 mg/kg bw/d) tris(2-chloroethyl)phosphate for a period of 30 days in the diet. No deaths occurred. At the end of the treatment no adverse effects on growth, appearance and behavior, liver and kidney weights, or changes in pathological examinations of survivors were seen.

Overall, no treatment-related effects in rats was shown up to 0.5% (approximately 250 mg/kg bw/d) tris(2-chloroethyl)phosphate in the diet for 30 days. The NOAEL was considered to be 250 mg/kg bw/d. Because of limitations in testing and reporting, this study should to be not useful for risk assessment.

18 months study

Information on neoplastic findings of tris(2-chloroethyl)phosphate in mice obtained from the following chronic toxicity study are described in detail in section 4.1.2.8. This 18 months dietary study in Slc:ddY mice was conducted as a part of a safety evaluation program on substitutes for fire proofing agents, especially to evaluate possible carcinogenicity. There were basic data comparable to the guideline OECD TG 451 with acceptable restrictions. The test parameters documented do not totally comply with this testing guideline for carcinogenicity. However, there was a well-documented study report which meets basic scientific principles. The supplied data are considered as valid for use in risk assessment.

Mice

Groups of 50 male and 50 female Scl:ddY mice received 0, 0.012, 0.06, 0.3, and 1.5% (equivalent to 0, 12, 60, 300, and 1500 mg/kg bw/d, calculated on an assumed body weight of

20 g and food consumption of 10% body weight/day) tris(2-chloroethyl)phosphate (purity 98%) by dietary administration for 18 months (Takada et al., 1989). Reduced survival was reported in male mice from week 70 and in female mice from week 60 compared to control groups. In both sexes fed 1500 mg/kg bw/d tris(2-chloroethyl)phosphate the final survival was approximately 40% compared to around 65% survival in controls. A marked reduction in body weight gain was noted in males and females receiving 1500 mg/kg bw/d (approximately 60% lower than the control value). No differences in food consumption were found between the groups for both sexes. In hematology, no changes were found apart from significant elevation in platelets in males of the 300 mg/kg bw/d dose group. Significant declines were found in the weights of the heart and testes in males and kidneys in females from the 1500 mg/kg bw/d dose group (no more data). At microscopy, various changes were observed in the different organs of all groups. The kidney and the liver were identified as target organs. There were predominate neoplastic changes in these organs (for details see 4.1.2.8 Carcinogenicity). Furthermore tris(2-chloroethyl)phosphate related non-neoplastic changes were reported from the liver and predominant from the kidney. In the liver, focal necrosis, vacuolation of the liver cells and extramedullary hematopoiesis were observed in animals of all groups including the control group. The most relevant effects were seen in the kidneys. In males and females of all treatment groups, hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei were observed. These nuclei were polymorphic, and several times abnormal division, degeneration and necrosis were observed. Unfortunately, no incidences of these findings were reported. However, such findings were not seen in the concurrent controls. In addition, cysts, necrosis of the urinary tubule epithelium and interstitial fibrosis were observed in animals receiving 1500 mg/kg bw/d.

In summary, the data reported for the highest dose level of 1500 mg/kg bw/d, e.g. reduced survival, reduction in body weight gain (>10%) and severe toxicity indicate that the top dose exceeds the MTD (maximum tolerated dose) for this mouse strain. However, relevant changes in tissue morphology were not only observed in male and female mice in the kidneys of the top dose level. Signs of prolonged regenerative cell proliferation, which occurs possibly after renal tubule damage were reported from all treatment groups. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei, which showed abnormal division, degeneration and necrosis at several times. Although no quantitative information on these microscopic findings in the kidney of treated groups were reported they were assessed as relevant toxic effects, also seen from this point of view that no such kidney findings were observed in the concurrent controls. Thus, a NOAEL for kidney effects (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) could not be established in this study. Therefore, 12 mg/kg bw/d is considered to be the LOAEL for kidney effects in male and female Scl:ddY mice in the 18 months diet study.

- *Inhalation*

No information available.

- *Dermal*

No information available.

4.1.2.6.1.2 Specific endpoint studies:

- *Neurotoxicity*

The neurotoxicity effects of tris(2-chloroethyl)phosphate were evaluated in White Leghorn hens only.

Hens

In an acute delayed neurotoxicity study according to OECD test guidelines 418 (no data on clinical biochemistry) the acute delayed neurotoxic potential of tris(2-chloroethyl)phosphate was determined in adult (12-14 months old), White Leghorn hens. 18 hens received two oral doses of 10 ml/kg (approximately 14200 mg/kg bw) tris(2-chloroethyl)phosphate (purity: commercial grade) with a three week interval (Stauffer Chemical Company, unpublished report 1979). Two additional groups, 10 hens per group, served as controls. A positive control group was treated with 500 mg/kg bw tri-o-cresyl phosphate, an organophosphorus neurotoxin. The negative control group received corn oil. Four out of 18 tris(2-chloroethyl)phosphate-treated hens died during the study. Predominant observed signs of toxicity were severe feather loss, cessation of egg production, reduced food consumption, and loss of body weight. Walking behavior of the tris(2-chloroethyl)phosphate-treated hens were identical to those seen in the corn oil group. There were no microscopical changes in brain, spinal cord or sciatic nerve. None of the 14/18 surviving hens receiving tris(2-chloroethyl)phosphate showed nerve fiber degeneration similar to that seen in tri-o-cresyl phosphate-treated positive controls.

No evidence of neurotoxicity was seen following two oral administrations (on Day 1 and again 3 weeks later) of 14.2 g/kg bw/d tris(2-chloroethyl)phosphate in hens.

- *Adverse effects on the brain in rats and mice after repeated exposure to tris(2-chloroethyl)phosphate*

Rats and mice

Clinical signs of neurotoxicity were observed in female F344/N rats during an oral 16-week gavage study and in B6C3F1 mice in the course of an oral range-finding toxicity study of 16 days. Clinical signs of toxicity, including ataxia, excessive salivation, gasping, and convulsion were noted in those female F344/N rats receiving double the target levels of 175 and 350 mg/kg bw/d tris(2-chloroethyl)phosphate for 3 days in week four during the 16-week toxicity study due to an incorrectly test material preparation. In addition in this subchronic study female rats receiving 175 and 350 mg/kg bw/d tris(2-chloroethyl)phosphate experienced occasional periods of hyperactivity after dosing. Periodic convulsions were noted in 350 mg/kg bw/d dosed females but not in males during week 12, and also not in the overdosed male rats in this study (NTP 1991, Matthews 1990). Male and female B6C3F1 mice given 350 and 700 mg/kg bw/d tris(2-chloroethyl)phosphate for 16 days exhibited ataxia and convulsive movements during the first 3 days of dosing (NTP 1991, Matthews 1990).

No adverse effects on growth, appearance and behavior were seen in the 2-year gavage studies with rats given 44 and 88 mg/kg bw/d of tris(2-chloroethyl)-phosphate and in the mice given up to 350 mg/kg bw/d (NTP 1991, Matthews 1993).

- *Determination of the acetylcholinesterase activity*

Since it is known that most organophosphates cause neurotoxic effects in humans through the inhibition of the enzyme acetylcholinesterase (AChE) in the central and peripheral nervous system, measurements of a possible inhibition of that enzyme as consequence to tris(2-chloroethyl)phosphate treatment in experimental animals were investigated in 14-day and 16-week oral studies in F344/N rats and B6C3F1mice (NTP 1991, Matthews 1990), and also in a three months dietary subchronic study in Sprague-Dawley CD rats (Stauffer Chemical Company, unpublished report 1980b). To assess the AChE inhibition in toxicity studies a multi-stage approach is indispensable. However, there are no data available for measurements the AChE inhibition in the CNS and PNS, in erythrocytes or whole blood. Only results of cholinesterase (ChE) measurements in serum are available. It has to be taken into consideration that testing serum cholinesterase is not very useful as the neuronal enzyme has different structural requirements for inhibition by organophosphates. Rats

Relevant treatment-related changes in serum cholinesterase activity were noted in female F344/N rats given 175 and 350 mg/kg bw/d tris(2-chloroethyl) phosphate for 16 weeks. Cholinesterase activity determined in serum at necropsy was down to 75% and 59% of the control value at 175 and 350 mg/kg bw/d, respectively ($p \leq 0.01$). No changes in activity were observed in treated male F344/N rats. The observed inhibition of cholinesterase activity is considered only as indication for exposure to TCEP.

- *Histologically examinations of the brain*

Reports in the literature indicate that most organophosphorous compounds induce neurotoxic effects. Therefore, the NTP data were included in a further investigation in order to characterize the effect of tris(2-chloroethyl)-phosphate on the nervous system. In these examinations additionally sections of forebrain, midbrain, brain stem and cerebellum from each animal were examined by light microscopy (Matthews 1990).

Rats and mice

In a 16-week subchronic oral (gavage) toxicity study in F344/N rats, tris(2-chloroethyl)phosphate-related neuronal necrosis occurred in the hippocampus and thalamus of female rats at 175 (8/10) and 350 mg/kg bw/d (10/10) and, to a lesser extent, in male rats treated at 350 mg/kg bw/d (2/10) (NTP 1991; Matthews 1990). At interim evaluation after 66 weeks of the 2-year study on F344/N rats, brain lesions were focally located in the cerebrum and thalamus. They were characterized by necrosis with accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia. Such lesions were observed in 3/10 females given 88 mg/kg bw/d. A marked increase in the incidence of degenerative lesions of the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia) such as

gliosis, hemorrhage, necrosis, and mineralization was noted in female F344/N rats at 88 mg/kg bw/d after oral long-term/long-life exposure (103 weeks). In contrast to the findings in the 16-week study, degenerative lesions were widely distributed in the gray and white matter of the brain stem and cerebral cortex of females receiving 88 mg/kg bw/d. Degenerative lesions of the brain occurred in the cerebrum and brain stem of more than 50% of female rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate; similar lesions were seen in only a few dosed male rats. In both sexes the brain lesions varied in severity from minimal to marked and often involved extensive areas. In some animals, the lesions were bilateral and symmetrical; in others, they were bilateral and asymmetrical or unilateral. The active lesions were characterized by degeneration and necrosis with hemorrhage, while resolving lesions exhibited loss of neurons and neutrophil, proliferation of glial cells, capillary hyperplasia, hypertrophy of the tunica media of small vessels, and hemosiderin-laden macrophages. Deposits of minerals occurred in some of these foci (NTP 1991, Matthews 1993). An overview of non-neoplastic brain lesions in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.10.

Table 4.10 Selected brain lesions in F344/N rats in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**44 mg/kg bw/d**	**88 mg/kg bw/d**
male (number examined)	50	49	50
Brain stem, hemorrhage			1(2%)
Brain stem, pigmentation, hemosiderin	1 (2%)		
Cerebrum, gliosis, focal			1 (2%)
Cerebrum, hemorrhage		1 (2%)	1 (2%)
Cerbrum, pigmentation, hemosiderin			1 (2%)
Pons, hemorrhage			3 (6%)
female (number examined)	50	50	50
Brain stem, gliosis	1 (2%)		15 (30%)**
Brain stem, hemorrhage	1 (2%)		12 (24%)**

Brain stem, mineralization			7 (14%)**
Brain stem, necrosis			1 (2%)
Brain stem, pigmentation, hemosiderin	1 (2%)		17 (34%)**
Cerebellum, hemorrhage	1 (2%)		2 (4%)
Cerebellum, necrosis			1 (2%)
Cerebellum, pigmentation, hemosiderin	1 (2%)		
Cerebrum, gliosis			19 (38%)**
Cerebrum, hemorrhage	1(2%)		17 (34%)**
Cerebrum, mineralization			15 (30%)**
Cerebrum, pigmentation, hemosiderin			22 (44%)**
Pons, hemorrhage		1 (2%)	

** significantly different ($p \leq 0.01$) from the control group by logistic regression tests

Mice were less sensitive to these effects to tris(2-chloroethyl)phosphate than rats. In B6C3F1 mice of both sexes no tris(2-chloroethyl)phosphate induced lesions of the brain were recorded at 44 to 700 mg/kg bw/d after repeated oral treatment by gavage for 16 weeks and up to and involving 350 mg/kg bw/d for 103 weeks (NTP 1991, Matthews 1993).

In summary no relevant toxic effects on the brain were observed following subchronic oral treatment of 175 mg/kg bw/d tris(2-chloroethyl)phosphate in male F344/N rats and of 88 mg/kg bw/d in females (NTP 1991; Matthews 1990). However, at the interim sacrifice after 66 weeks of the 2-year study F344/N rats, focal brain lesions in the cerebrum and thalamus were seen in females receiving 88 mg/kg bw/d, but not in males. After 103 treatment weeks such lesions in the brain were observed in over 50% of female F344/N rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate. Similar lesions occurred only in a few males, however, they were assessed as toxicologically relevant, although these findings were not significant increased in incidence and severity, but they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Therefore, the NOAEL for brain lesions is 44 mg/kg bw/d for male and female F344/N rats (NTP 1991; Matthews 1993). There were no tris(2-chloroethyl)phosphate related microscopic changes in the brain of B6C3F1 mice treated up to and involving 350 mg/kg bw/d for 103 weeks when compared with controls. Therefore, the NOAEL for brain lesions is 350 mg/kg bw/d for B6C3F1 mice of both sexes (NTP 1991; Matthews 1993).

4.1.2.6.2 Human data

Ingerowski & Ingerowski (1997) reported a case of exposure to TCEP by a 5 year old child showing neurotoxic signs after a long term contact. The same case has been reported to the

Case Register of Federal Institute for Risk Assessment (formerly BgVV) (Case Register BgVV 1061/97), too.

The girl developed a progredient paresis. She slept in a room which was equipped with wood panellings treated with wood preserver containing 3% of TCEP. Measurements of TCEP in the timber work revealed 600 mg/kg of wood. 9 further chemicals were also analysed but below the detection limit. EMG and nerve conduction velocity testing showed generalized neurogenic defects, whereas the clinical findings could not be clearly classified as "spinal muscle dystrophy". In the further clinical course a diagnosis of "spinal muscle dystrophy of Kugelberg-Welander type" was established with tetraparesis. After renovation of the house (removal of all timber panels), the clinical status improved. Two years after exposure no functional deficits are present.

Because TCEP was not determined in house dust, an exposure analysis based on the concentration in the wood panel only identifies the possible source, but does not help for quantifying the exposure. In this case, oral exposure is possible by uptake of dust and inhalation exposure inhaling particles containing TCEP. Dermal contact should also be taken into account by contact with dust and touching the panels. The symptoms of illness increased with increasing time of exposure, and ceased after discontinuation (dechallenge reaction). From these reasons, a relationship between TCEP exposure and the neurotoxic signs cannot be neglected.

4.1.2.6.3 Summary of animal toxicity data after repeated exposure to tris(2-chloroethyl)phosphate

The main toxic effects of tris(2-chloroethyl)phosphate from animal toxicity studies were summarized in the following table, Table 4.11.

Table 4.11:

Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
F344/N rat (5m/5f) Oral Gavage 16 days, 5 d/wk (12 doses) 0, 22, 44, 88, 175, 350 mg/kg bw/d	350 mg/kg bw: ↑** liver weight (f) ≥175 mg/kg bw/d: ↑** kidney weight, abs/rel (m) ↓** serum cholinesterase activity (f) ≥88 mg/kg bw/d: ↓* lung weight, abs/rel (f) $NOAEL_{sys}$ (m/f): 350 mg/kg bw/d	NTP 1991 Matthews 1990
F344/N rat (10m/10f) Oral Gavage 16/18 weeks (f/m), 5 d/wk 0, 22, 44, 88, 175, 350 mg/kg bw/d	350 mg/kg bw/d: mortality: 4/10 (m), 3/10 (f) periodic convulsion during week 12 (f) ↑** liver and kidney weights, rel (m) ↓ brain, thymus, abs (f) neuronal necrosis, loss of neurons in the brain (f:10/10; m: 2/10) ≥175 mg/kg bw/d: in the brain: neuronal necrosis (10/10 f) loss of neurons (8/10 f) ↓** serum cholinesterase activity (f) ≥44 mg/kg bw/d: ↑** liver and kidney weights, rel (f) $NOAEL_{sys}$ for brain lesions: (m): 175 mg/kg bw/d (f): 88 mg/kg bw/d	NTP 1991 Matthews 1990
F344/N rat (10m/10f) Oral Gavage 66 weeks (interim sacrifice), 5 d/wk 0, 44, 88 mg/kg bw/d rat	88 mg/kg bw/d: ↓** AP (f), ↓** ALAT (f) ↑** liver and kidney weights, rel (m) renal tubule adenoma (1/10m) brain: local necrosis, accumulation of inflammatory cells, reactive gliosis, endothelial hypertrophy (3/10 f) $NOAEL_{sys}$ for brain lesions (f): 44 mg/kg bw/d	NTP 1991 Matthews 1993

F344/N rat (60m/60f) Oral Gavage 103 weeks, 5 d/wk 0, 44, 88 mg/kg bw/d rat	88 mg/kg bw/d: ↓** survival (m/f); ↑** focal hyperplasia of tubule epithelium of the kidney (m:24/50; f: 16/50) ↑** degenerative lesions in the brain (f) ↑ lesions in the brain (m) 44 mg/kg bw/d: ↑** focal hyperplasia of tubule epithelium of the kidney (m:2/50; f: 3/50) $LOAEL_{sys}$ for kidney lesions (m/f): 44 mg/kg bw/d $NOAEL_{sys}$ for brain lesions (m/f): 44 mg/kg bw/d	

For key to symbols, see at the end of the table

Table 4.11 (continued):

Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
Sprague-Dawley CD Rat (10m/10f) Oral In feed 28 days, daily 0, 500, 850, 1500, or 2000 ppm (m: 0, 41.75, 71.5, 125 or 163 mg/kg bw/d; f: 0, 50, 88, 144 or 191 mg/kg bw/d); 200→4000 ppm (m: 293 mg/kg bw/d; f: 334 mg/kg bw/d); 350 ppm (m: 30 mg/kg bw/d; f: 38 mg/kg bw/d)→8000 ppm (m: 495 mg/kg bw/d; f: 508 mg/kg bw/d)	8000 ppm (m: 495 mg/kg bw/d; f: 508 mg/kg bw/d): ↓** food consumption (after 1 week) smaller seminal vesicles and/or prostate (m: 3/10) ↓** testes weight, size (m: 1/10) 1500 ppm (m: 125 mg/kg bw/d): smaller seminal vesicles and/or prostate (m: 1/10) $NOAEL_{sys}$ (m): 850 ppm (71.5 mg/kg bw/d) $NOAEL_{sys}$ (f): 8000 ppm (508 mg/kg bw/d)	Stauffer Chemical Company 1980a
Sprague-Dawley CD Rat (20m/20f) Oral in feed 3 months, daily 0, 400, 1000, 3000, or 8000 ppm (m: 0, 25.96, 65.43, 192, or 506 mg/kg bw/d; f: 0, 30.4, 75.15, 215, or 586 mg/kg bw/d)	8000 ppm (m: 506 mg/kg bw/d; f: 586 mg/kg bw/d): ↓* food consumption (m/f) ↓* body weight (m/f) ↑* relative liver and kidney weights (m/f) ↑ incidence in regenerative hyperplasia in the renal cortex 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d): ↓ food consumption (f) ↓ body weight (m/f) ↑* relative liver and kidney weights (m/f) 1000 ppm (75 mg/kg bw/d): ↑* relative kidney weights (m) $NOAEL_{sys}$: (m/f): 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) for kidney effects	Stauffer Chemical Company 1980b
Sprague-Dawley Rat (10m/10f) Oral In feed 30 days, daily 0.5% (250 mg/kg bw/d)	5000 ppm (appr. 250 mg/kg bw): no treatment-related effects $NOAEL_{sys}$ (m/f): 5000 ppm (appr. 250 mg/kg bw)	Ulsamer 1980

For key to symbols, see at the end of the table

Table 4.11 (continued):
Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
B6C3F1 Mice (5m/5f) Oral Gavage 16 days, 5 d/wk (12 doses) 0, 44, 88, 175, 350, or 700 mg/kg bw/d	≥350 mg/kg bw: ataxia, convulsive movements during the first three days of dosing (m/f) $NOAEL_{sys}$ (m/f): 175 mg/kg bw/d	NTP 1991 Matthews 1990
B6C3F1 Mice (10m/10f) Oral Gavage 16 weeks (f), 18 weeks (m), 5 d/wk 0, 44, 88, 175, 350, or 700 mg/kg bw/d	700 mg/kg bw/d: ↑** liver weights, abs (m) ↑** nuclear enlargement of tubule epithelial cells of the kidney (10/10 m/f) ↓* sperm count (10/10 m) ≥175 mg/kg bw/d: ↑** absolute liver weights (f) ↓** absolute kidney weights (m) $NOAEL_{sys}$ for renal lesions (m/f): 350 mg/kg bw/d	NTP 1991 Matthews 1990
B6C3F1 Mice (10m/10f) Oral Gavage 66 weeks (interim sacrifice), 5 d/wk 0, 175, 350 mg/kg bw/d	350 mg/kg bw/d: hyperplasia of tubule epithelial cells of the kidney (2/10 m) $NOAEL_{sys}$ (m/f): 175 mg/kg bw/d	NTP 1991 Matthews 1993
B6C3F1 Mice (60m/60f) Oral Gavage 103 weeks, 5 d/wk 0, 175, 350 mg/kg bw/d rat	350 mg/kg bw/d: ↑** nuclear enlargement of tubule epithelial cells of the kidney (39/50 m; 44/50 f) 175 mg/kg bw/d: ↑** nuclear enlargement of tubule epithelial cells of the kidney (16/50 m; 5/49 f) $LOAEL_{sys}$ for kidney lesions (m/f): 175 mg/kg bw/d $NOAEL_{sys}$ not derived for kidney lesions	
Scl:ddY mice (50m/50f) Oral in feed 18 months, daily 0, 12, 60, 300, 1500 mg/kg bw/d	1500 mg/kg bw/d: cysts, necrosis of the urinary tubule epithelium and interstitial fibrosis ≥12 mg/kg bw/d: hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei $LOAEL_{sys}$ for kidney effects (m/f): 12 mg/kg bw/d $NOAEL_{sys}$ not derived for kidney lesions	Takada et al. 1989

White Leghorn Hens (18 test animals, 10/negativ and 10/positiv control group) Oral by stomach tube 2 treatments (on day 1 and again 3 weeks later) 0, 14200 mg/kg bw	14200 mg/kg bw: mortality (4/18) ↓** body weight cessation of egg production feather loss $NOAEL_{sys}$: not derived	Stauffer Chemical Company 1979

↑**: statistically significant increase compared with controls ($p<0.01$); ↑*: statistically significant increase compared with controls ($p<0.05$);↑ increase compared with controls , no statistically significant but possibly of toxicological relevance; ↓**: statistically significant decrease compared with controls ($p<0.01$); ↓*: statistically significant decrease compared with controls ($p<0.05$); ↓: decrease compared with controls, no statistically significant but possibly of toxicological relevance; →: during the study dose level was increased to; m: male; f: female; AP: Alkaline phosphatase; ALAT: Alanine aminotransferase; abs: absolute; rel: relative; $LOAEL_{sys}$: lowest observed adverse effect level for systemic effects; $NOAEL_{sys}$: no observed adverse effect level for systemic effects

In experimental animals, the primary target organs after short- and long-term oral exposure to tris(2-chloroethyl)phosphate were the brain, the kidneys, and the liver.

Effects in the brain:

In rats, but not in mice, long-term oral exposure (subchronic and chronic) of tris(2-chloroethyl)phosphate caused degenerative lesions consisting of gliosis, mineralization, hemorrhage, and pigmentation (hemosiderin accumulation) in the cerebrum and brain stem. These degenerative lesions in the brain of rats occurred in a dose related pattern, and in addition, in a clear time-response relationship in frequency, intensity and severity. Male rats are less sensitive to adverse brain effects than female rats. Degenerative lesions in the brain were observed in female F344/N rats at ≥175 mg/kg bw/d after a treatment period of 16 weeks and after a long-term exposure in more than 40% of female F344/N rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate. Similar lesions were seen in male F344/N rats at 350 mg/kg bw/d after treatment for 16 weeks and in only a few long-term dosed male F344/N rats at 88 mg/kg bw/d. Although these findings in the brain of long-term dosed male F344/N rats were not significant increased in incidence and severity, they were assessed as toxicologically relevant, because they were elevated in a small number in relation to treatment with tris(2-chloroethyl)phosphate when compared with control data (NTP 1991, Matthews 1990). In B6C3F1 mice treated at 44 mg/kg bw/d to 700 mg/kg bw/d tris(2-chloroethyl)phosphate by gavage for 16 weeks and up to and involving 350 mg/kg bw/d for 103 weeks no such microscopic changes in the brain were observed (NTP 1991, Matthews 1993).

A relevant reduction of serum cholinesterase activity was determined only in female F344/N rats receiving ≥175 mg/kg bw/d tris(2-chloroethyl)phosphate by gavage for 16 weeks (NTP 1991, Matthews 1990). No inhibition of cholinesterase activity were determined in tris(2-chloroethyl)phosphate-treated male F344/N rats (NTP 1991, Matthews 1990), and also not in male and female Sprague-Dawley CD rats receiving dietary doses of up to 8000 ppm (equivalent to about 506 mg/kg bw/d in males and 586 mg/kg bw/d in females) for three months (Stauffer Chemical Company, unpublished report 1980). In B6C3F1 mice of both sexes receiving each up to 700 mg/kg bw/d tris(2-chloroethyl)phosphate serum cholinesterase activity was similar to that of controls both after subacute and subchronic oral administration (NTP 1991, Matthews 1990). Since no measurements to AChE inhibition in the CNS, PNS, and in erythrocytes are carried out, it is difficult to assess the results of ChE measurements in plasma. For this reason the observed inhibition of plasma ChE is considered only as indicator for exposure to tris(2-chloroethyl)phosphate.

A very high oral dose of tris(2-chloroethyl)phosphate (14200 mg/kg bw) did not cause behavioral effects or nerve damage suggestive of neurotoxicity in White Leghorn hens (Stauffer Chem. Company 1979).

Effects in the kidneys:

Differences in kidney weights when compared with that of controls were presented in studies on rats and mice after repeated exposure to tris(2-chloroethyl)phosphate. In Sprague-Dawley CD rats repeated oral administration via the diet for three months of ≥1000 ppm (equivalent to 65.43 mg/kg bw/d) in males and of ≥3000 ppm (equivalent to 214.62 mg/kg bw/d) in females led to a significant increase of the mean relative kidney weights (Stauffer Chem. Company 1980). Increased relative kidney weights were also observed in female F344/N rats

receiving ≥44 mg/kg bw/d by gavage for 16 weeks (NTP 1991, Matthews 1990). In male F344/N rats the relative kidney weights were significantly increased at 350 mg/kg bw/d after gavage for 16 weeks (NTP 1991, Matthews 1990) and of both relative and absolute kidney weights at 88 mg/kg bw/d after 66 weeks (NTP 1991, Matthews 1993). Histopathologic examination of these kidneys showed an increase in incidence in regenerative hyperplasia in the renal cortex for male Sprague-Dawley CD rats receiving 8000 ppm (approximately 506 mg/kg bw/d) tris(2-chloroethyl)phosphate in the diet for three months (Stauffer Chem. Company 1980b). Markedly increase of focal or multifocal hyperplasia of the renal tubule epithelium was observed in male and female F344/N rats receiving 44 or 88 mg/kg bw/d for 103 weeks (NTP 1991; Matthews 1993). In B6C3F1 mice, reduced absolute kidney weights were determined in males receiving ≥175 mg/kg bw/d after repeated oral administration by gavage of tris(2-chloroethyl)phosphate for 16 weeks. At 700 mg/kg bw/d mild cytomegaly of the epithelium of the proximal tubules and loops of Henle was observed in kidneys of B6C3F1 mice of both sexes (NTP 1991, Matthews 1990). After long-life exposure, karyomegaly of renal tubule epithelial cells was seen in male and female B6C3F1 mice treated with ≥175 mg/kg bw/d and was present in approximately 80% of the 350 mg/kg bw dosed B6C3F1 mice (NTP 1991; Matthews 1993). In a further study in mice performed to determine the carcinogenic potential of tris(2-chloroethyl)phosphate relevant changes in the tissue morphology of kidneys were observed. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei. Those findings were also seen in the kidneys of male and female Scl:ddY mice fed ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months, but were not reported from the concurrent control groups. In mice receiving 1500 mg/kg bw/d, additionally necrosis of the urinary tubule epithelium and interstitial fibrosis were observed (Takada et al., 1989).

Effects in the liver:

In female F344/N rats receiving 350 mg/kg bw/d by gavage for 16 days relative and absolute liver weights were significantly (17%) increased when compared to controls (Matthews 1990; NTP 1991). At the same dose level increased relative liver weights were observed in male F344/N rats treated for 16 weeks and in females at ≥44 mg/kg bw/d, respectively. After 66 weeks mean absolute and relative liver weights were significantly increased in male F344/N rats receiving 88 mg/kg bw/d (Matthews 1990, NTP 1991). In male and female Sprague-Dawley CD rats repeated oral administration of ≥3000 ppm (equivalent to ≥191.87 mg/kg bw/d in males and ≥214.62 mg/kg bw/d in females) via the diet for three months showed a significant increase of the mean relative liver weights (Stauffer Chem. Company 1980). In B6C3F1 mice the mean absolute liver weights were significantly ($p \leq 0.01$) increased in females receiving 175 to 700 mg/kg bw/d and in male mice receiving 700 mg/kg bw/d (Matthews 1990, NTP 1991). Changes in liver morphology were noted in Scl:ddY mice of all dose groups (≥12 mg/kg bw/d). There were focal necrosis, vacuolation of hepatocytes and extramedullary hematopoiesis. However, such findings were also observed in controls.

Effects in the liver observed in F344/N rats and B6C3F1 mice after treatment with tris(2-chloroethyl)phosphate were limited to increased organ weights, without other corroborating findings (morphology or biochemistry). Therefore, these findings in organ weights were not considered to be toxicologically adverse.

No tests for repeated dose toxicity by inhalation on experimental animals are available. There is no toxicity study in conformity to the current requirements of the current repeated dose toxicity testing protocols by the dermal application route. No firm conclusion could be drawn from short and long-term skin tests for initiating/promoting activity in Swiss mice described in section 4.1.2.8.

No/Lowest observed adverse effect level (N/LOAEL)

The data submitted are considered acceptable with regard to the basic requirements as specified in Annex VIIA of Directive 67/548/EEC. The available data permit the derivation of $NOAEL_{sys}$ for repeated-dose oral toxicity. No studies are available to assess toxicity after repeated inhalation and dermal exposure.

Oral exposure:

In experimental animals, the principal affected organs observed following repeated oral administration are CNS, kidney and liver. However, relevant toxic effects were observed in the brain and kidneys.

The $NOAEL_{sys}$ values deriving from animal studies to tris(2-chloroethyl)phosphate after oral administration for brain and kidney findings after different exposure duration are summarized in Table 4.12.

Table 4.12

Summary table: $NOAEL_{sys}$ values for relevant non-neoplastic effects in the brain and kidneys derived from animal studies to tris(2-chlorethyl)phosphate

Species, strain (m/f)	Exposure route	Exposure duration	$NOAEL_{sys}$ for relevant non-neoplastic effects	Reference
F344/N rat (5m/5f)	Oral by gavage	16 days 5 d/wk	m/f: 350 mg/kg bw/d m: for brain and kidney effects	NTP 1991 Matthews 1990
F344/N rat (10m/10f)	Oral by gavage	16 weeks 5 d/wk	f: 88 mg/kg bw/d m: 175 mg/kg bw/d for neurotoxicological effects	NTP 1991 Matthews 1990
Sprague-Dawley CD rat (10m/10f)	Oral in feed	3 months daily	m/f: 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) for kidney effects	Stauffer Chemical Company 1980b
F344/N rat (60m/60f)	Oral by gavage	103 weeks 5 d/wk	m/f: 44 mg/kg bw/d for brain lesions m/f: not established for kidney effects	NTP 1991 Matthews 1993
B6C3F1 mouse (5m/5f)	Oral by gavage	16 days 5 d/wk	m/f: 175 mg/kg bw/d for convulsion and ataxia	NTP 1991 Matthews 1990
B6C3F1 mouse	Oral by gavage	16 weeks 5 d/wk	m/f: 350 mg/kg bw/d for kidney effects	NTP 1991 Matthews

Species, strain (m/f)	Exposure route	Exposure duration	$NOAEL_{sys}$ for relevant non-neoplastic effects	Reference
(10m/10f)				1990
B6C3F1 mouse (10m/10f)	Oral by gavage	66 weeks 5 d/wk	m/f: 350 mg/kg bw/d for brain lesions m/f: 175 mg/kg bw/d for kidney lesions	NTP 1991 Matthews 1993
B6C3F1 mouse (60m/60f)	Oral by gavage	103 weeks 5 d/wk	m/f: 350 mg/kg bw/d for brain lesions m/f: not established for kidney effects	NTP 1991 Matthews 1993
Scl:ddY mice (50m/50f)	Oral in feed	18 months, daily	m/f: not established for kidney effects	Takada et al. 1989

m: male; f: female; $NOAEL_{sys}$: No observed adverse effect level for systemic effects

Derivation of a NOAEL for brain effects:

Tris(2-chloroethyl)phosphate produces brain lesions in female F344/N rats and, to a lesser extent in male rats, but not in mice (NTP 1991, Matthews 1990). They were observed in several guideline studies with different duration. In female F344/N rats, neurotoxicity was induced at dosages of ≥175 mg/kg bw/d tris(2-chloroethyl)phosphate after an exposure period of 16 weeks and the absence of these lesions was found at 88 mg/kg bw/d. However, 88 mg/kg bw/d tris(2-chloroethyl)phosphate induced degenerative lesions in the brain of female rats after a life-time exposure. In male F344/N rats similar lesions were seen after treatment for 16 weeks at 350 mg/kg bw/d, and in very few cases at 88 mg/kg bw/d after 103 treatment weeks. Although these findings in the brain on male F344/N rats at 88 mg/kg bw/d were not significant increased in incidence and severity, they were assessed as toxicologically relevant, because they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Therefore, a no observed adverse effect level (NOAEL) of 44 mg/kg bw/d for brain lesions could be derived for male and female F344/N rats from the 103-week (gavage) rat study (NTP 1991, Matthews 1993). Mice were less sensitive for brain effects to tris(2-chloroethyl)phosphate than rats. There were no tris(2-chloroethyl)phosphate related microscopic changes in the brain of B6C3F1 mice treated up to and involving 350 mg/kg bw/d for 103 weeks when compared with controls. Therefore, the NOAEL for brain lesions is 350 mg/kg bw/d for B6C3F1 mice of both sexes (NTP 1991, Matthews 1993). A very high oral dose of tris(2-chloroethyl)phosphate (14200 mg/kg bw) did not cause behavioral effect or nerve damage suggestive of neurotoxicity in White Leghorn hens (Stauffer Chem. Company 1979).

For adverse effects on the brain (hippocampal lesions):

103-week oral (gavage)/ F344/N rat

$NOAEL_{sys}$ 44 mg/kg bw/d (NTP 1991; Matthews 1993)

103-week oral (gavage)/ B6C3F1 mice

$NOAEL_{sys}$ 350 mg/kg bw/d (NTP 1991; Matthews 1993)

Derivation of a N/LOAEL for kidney effects:

Kidney effects were observed in rats and mice each of both sexes in several studies with different duration. The studies were guideline studies or comparable to guideline studies with acceptable restrictions. Changes in the tissue morphology of the kidneys appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl)phosphate in rats and mice, especially in context with carcinogenesis. A NOAEL for these kidney findings could not be derived for male and female F344/N rats and for B6C3F1 mice of both sexes in chronic toxicity studies. In F344/N rats, mostly findings in the kidneys appeared at doses ≥44 mg/kg bw/d tris(2-chloroethyl)phosphate. At this dosage an increase of relative kidney weights was observed in female F344/N rats after a 16-week repeated oral administration by gavage, and focal hyperplasia of tubule epithelium of the kidney in male and female F344/N rats after life-time oral (gavage) administration (NTP 1991, Matthews 1990). In a feeding study of 3 months statistically significant increase in relative kidney weight was noted in male Sprague-Dawley CD rats at doses of 1000 ppm (approx. 65 mg/kg bw) and above and in female rats at 3000 ppm (approx. 215 mg/kg bw/d) and above. In male rats treated with 8000 ppm (approximately 506 mg/kg bw/d) tris(2-chloroethyl)phosphate an increased incidence in regenerative hyperplasia in the renal cortex was observed and a NOAEL for kidney effects for male and female Sprague-Dawley CD rats at 3000 ppm (equivalent to 192 mg/kg bw/d in males; and 215 mg/kg bw/d in females) (Stauffer Chemical Company 1980b). In a well-conducted 16-week study by gavage in B6C3F1 mice, a no observed adverse effect level of 350 mg/kg bw/d for mice of both sexes was derived (NTP 1991, Matthews 1990). However, after long-term exposure to 175 mg/kg bw/d and 350 mg/kg bw/d tris(2-chloroethyl)phosphate, karyomegaly of renal tubule epithelial cells was noted in male and female B6C3F1 mice given in the low dose 175 mg/kg bw/d and was present in approximately 80% of the 350 mg/kg bw/d dosed male and female mice. Because a NOAEL for kidney lesions could not be established for male and female B6C3F1mice in the 2-year study, 175 mg/kg bw/d is considered to be the LOAEL for kidney lesions in B6C3F1 mice of both sexes (NTP 1991, Matthews 1993). Kidney lesions observed as hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei were noted in male and female Scl:ddY mice fed at ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months. Unfortunately, no incidences on the microscopic findings in the kidneys of treated groups were reported. There are no published historical control data for the ScI:ddYY mouse strain. However, there are data from concurrent control groups of a 2-year study investigated the chronic toxicity and the carcinogenicity of 1,2,4-trichlorbenzene on the skin using the ScI:ddYY mouse strain (Yamamoto, 1982). In the control groups of 50 male and 50 female mice skin-painting was carried out with acetone alone. No findings of cellular necrosis with a regenerative hyerplasia of the urinary tubule epithelium or enlargement of the nuclei were reported in this study from the concurrent controls. As main microscopic findings in the kidneys inflammation and amylolidosis were observed. The following incidences were noted: inflammation in 12/50 males and in 2/50 females; amyloidosis in 4/50 males and in 5/50 females.

In addition in the 18-month dietary study, changes in tissue morphology of the kidneys were not only observed in male and female mice at the top dose level of 1500 mg/kg bw/d, which clear exceeded the MTD (maximum tolerated dose) for this mouse strain. Sign of cytotoxicity leading to cellular necrosis with a regenerative hyperplasia were reported from all treatment groups. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei, which showed abnormal division, degeneration and necrosis at several times. Although no quantitative information on these microscopic findings in the kidney of treated groups were reported they were assessed as relevant toxic effects, also seen from this point of view that no such kidney findings were observed in the concurrent controls. Thus, 12 mg/kg bw/d is considered to be the LOAEL for kidney lesions in Scl:ddY mice of both sexes (Takada et al., 1989).

For kidney lesions:
103-week oral (gavage)/ F344/N rat

$LOAEL_{sys}$ 44 mg/kg bw/d (NTP 1991, Matthews 1993)

3-months oral (feed)/male Sprague-Dawley CD rat
$NOAEL_{sys}$ 3000 ppm (192 mg/kg bw/d) (Stauffer Company 1980b)

103-week oral (gavage)/ B6C3F1 mice
$LOAEL_{sys}$ 175 mg/kg bw/d (NTP 1991, Matthews 1993)

18 months oral (feed)/ Scl:ddY mice
$LOAEL_{sys}$ 12 mg/kg bw/d (Takada et al., 1989)

Most of the available studies in rats and mice also reported effects in the liver. These findings observed in F344/N rats and B6C3F1 mice were limited to increased organ weights, without other corroborating findings in morphology. Therefore, these findings in organ weights were not considered to be toxicologically relevant. However, changes in liver morphology were reported in Scl:ddY mice of all dose groups (≥12 mg/kg bw/d). There were focal necrosis, vacuolation of hepatocytes and extramedullary hematopoiesis. However, such findings were also observed in controls.

In conclusion, all listed studies were of sufficient data quality. Degenerative lesions in the brain occurred in rats in a dose related pattern, and in addition, in a clear time-response relationship in frequency, intensity and severity. Male rats were less sensitive to adverse brain effects than female rats. The lowest NOAELs for systemic effects/brain effects were derived in each case from subchronic and chronic oral toxicity studies for rats at dose levels between 44 to 88/175 mg/kg bw/d. Mice were less sensitive to tris(2-chloroethyl)phosphate than rats with respect to brain effects. NOAELs for brain effects were established between 175 to 350 mg/kg bw/d in mice of both sexes.

However, the most sensitive NOAEL/LOAEL was derived for kidney lesions. Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl)phosphate and both the rat and the mouse are the species most sensitive to tris(2-chloroethyl)phosphate. Kidney effects observed in rats and mice were dose- and time-related

with respect to incidence and severity. Changes in kidney morphology were noted in Sprague-Dawley CD rats and F344/N rats and in B6C3F1 and Scl:ddY mice. There were hyperplasia, and karyomegaly in the cortical tubule epithelium in the kidneys combined with signs of cellular necrosis. In both rat strains and both mouse strains kidney lesions of this same kind were observed. Only in subchronic toxicity studies a no-observed-adverse-effect-level (NOAEL) for kidney lesions was estimated; for male and female Sprague-Dawley CD rats at 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) (Stauffer Chemical Company 1980b) and for B6C3F1 mice at 350 mg/kg bw/d (NTP 1991, Matthews 1990). However, a NOAEL for tissue changes in the kidney could not be derived for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d tris(2-chloroethyl)phosphate, furthermore, for B6C3F1 mice after long-life exposure to ≥175 mg/kg bw/d (NTP 1991, Matthews 1993), and also not for Scl:ddY mice after feeding of ≥12 mg/kg bw/d in the diet for 18 months (Takada et al. 1989). This carcinogenicity study in Scl:ddY mice (cf. 4.1.2.8.1) differs in some respects from the published guidelines, however, the study is accepted due to the fact that the test procedure described is comparable to the guideline study with acceptable restrictions and is performed in accordance with generally accepted scientific standard. Therefore, the lowest LOAEL of 12 mg/kg bw/d for kidney lesions in Scl:ddY mice will be chosen as the basis for risk characterisation.

No data on local effects after repeated exposure to TCEP are available.

Conclusion on classification for repeated dose studies:

On the basis of the available data, classification of tris(2-chloroethyl)phosphate as harmful and labelling with Xn, R 48/22 (Harmful: danger of serious damage to health by prolonged exposure if swallowed) according to the criteria given in Directive 67/548/EEC is not necessary.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Bacterial assays (table 4.13)

In bacterial gene mutation tests there were negative results in Salmonella typhimurium tester strains TA 100, TA 1535 and TA 1538 up to approximately 10,000 µg/plate and in TA 98, TA 1537, up to 3333 µg/plate (Stauffer Chemical Company 1976; Prival et al. 1977; Haworth et al. 1983; NTP 1991).
Only Nakamura et al. (1979) reported on a positive finding with S-9 mix in one tester strain (TA 1535) for doses from 280 µg/plate up to 2800 µg/plate; higher doses had strong toxic effects.

Assays with mammalian cells (tables 4.14-4.17)

A mammalian cell mutation assay with mouse lymphoma cells was negative with and without S-9 mix up to a cytotoxic dose of 1.07 µl/ml (Stauffer Chem. 1978). A gene mutation assay with V79 cells, only done without S-9 mix, was negative for concentrations up to 2000 µg/ml; no informations about cytotoxicity were given (Sala et al. 1982).

A chromosomal aberration assays with Chinese hamster ovary cells were negative for concentrations up to 1600 µg/ml with and without S-9 mix. No informations about cytotoxic effects were given (Galloway et al. 1987: also citated in NTP 1991).

A SCE test with V79 cells (Sala et al., 1982) was marginally positive with S-9 mix at the highest tested dose of 700 µg/ml and without S-9 mix from 700 µg/ml up to 3000 µg/ml (with S-9 mix: 9.7 SCE per cell as compared to 7.1 in the control; without S-9 mix: up to 9.0 SCE per cell as compared to 5.5 in the control); without S-9 mix the highest tested dose was strongly toxic. Another SCE test, also done with and without S-9 mix, had an equivocal result. Only in one out of two experiments there was a slight increase of SCE frequencies with S-9 mix from 500 µg/ml up to 1600 µg/ml; without S-9 mix concentrations up to 160 µg/ml were negative (Galloway et al. 1987). No informations about toxic effects were given.

An UDS test (liquid scintillation counting) with human WI-38 cells was negative with S-9 mix for concentrations up to 0.5 µl/ml and without S-9 mix for concentrations up to 0.1 µl/ml (Stauffer Chem. 1979). No informations about toxic effects were given.

4.1.2.7.2 Studies in vivo

Rodent bone marrow micronucleus tests (table 4.18)

In two in vivo micronucleus tests negative results were observed in bone marrow cells of mice after oral administration or intraperitoneal injection of the tested substance up to doses which correspond to the maximum tolerated dose.
Otto (1984) reported on a negative finding after oral administration of 1000 mg/kg bodyweight. The tested dose induced clinical signs and lethal effects. There are no data on local toxicity (P/N ratio). In an IRI study (1993) a negative result was obtained after intraperitoneal injection of doses up to 700 mg/kg bodyweight. Toxic signs were observed from 350 mg/kg bodyweight upwards. The highest tested dose of 700 mg/kg bodyweight induced lethal effects and at the 48 h-sampling also local cytotoxic effects (decrease of P/N ratio).

Furthermore, Sala et al. (1982) reported on an in vivo micronucleus assay with Chinese hamsters. After single intraperitoneal injection of doses up to 250 mg/kg bodyweight approximately a doubling of the spontaneous frequency of micronucleated polychromatic erythrocytes was found at 125 and 250 mg/kg bodyweight. Although a slight increase of micronucleated cells was observed the overall result was characterized as questionable by the authors. The authors also concluded that further data in the same or in other species are needed.

Drosophila melanogaster (table 4.19)

Vogel and Nirvard (1991) reported on a negative test for mitotic recombination in somatic cells of Drosophila (test solutions up to 40 mmol/l were dropped on the surface of the food).

4.1.2.7.3 Conclusion

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, in a mouse-lymphoma-assay and in a UDS-test. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Two in vivo mice micronucleus tests were negative for application up to maximum tolerated doses, whereas a positive result of questionable validity was observed in another test. Also a Drosophila test was negative.
Overall, it can be concluded that there is no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate.

Table 4.13 In vitro tests: Bacterial genotoxicity

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Gene mutation; TA,98, TA 100, TA 1535, TA 1537, TA 1538	0.001 - 1.0 µl/plate (1.0 - 1000 µg/plate)	0.001 - 1.0 µl/plate (1.0 - 1000 µg/plate)	negative	no toxic effects	plate incorporation method purity: no data	Stauffer Chemical Company, 1976
Gene mutation; TA 100, TA 1535, TA 1538	1.0 - 10 µl/plate (1000 - 10'000 µg/plate)	1.0 - 10 µl/plate (1000 - 10'000 µg/plate)	negative	no toxic effects	plate incorporation method purity: no data	Prival et al., 1977
Gene mutation; TA 98, TA 100, TA 1535, TA 1537	33 - 3333 µg/plate	33 - 3333 µg/plate	negative	with and without S-9 mix at the highest tested dose	preincubation method purity: 99.5 %	Haworth et al., 1983
Gene mutation; TA 98, TA 100, TA 1537, TA 1538	330 - 3330 µg/plate	33 - 3333 µg/plate	negative	with and without S-9 mix at the highest tested dose	preincubation method purity: 98.0 %	NTP, 1991
Gene mutation; TA 98, TA 100, TA 1535, TA 1537, TA 1538	1.0 - 30 µmol/plate (285.5 - 8565 µg/plate)	1.0 - 30 µmol/plate (285.5 - 8565 µg/plate)	positive	with and without S-9 mix at the highest tested dose	strongly positive only in TA 1535 from 1.0 µmol/plate up to 10 µmol/plate (higher doses were strongly toxic); dose-dependent effect purity: no data	Nakamura et al., 1979

Table 4.14 In vitro tests: Mammalian cell gene mutations or Mouse-lymphoma-assay

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			

mouse lymphoma assay; L5178Y cells; tk locus	0,09 - 1.07 µl/ml (90 - 1070 µg/ml)	0,09 - 1.07 µl/ml (90 - 1070 µg/ml)	negative	toxicity: with S-9 mix clear effect at the highest tested dose; without S-9 mix clear effects from 0.8 µl/ml upwards purity: no data	Stauffer Chemical Company, 1978
HPRT locus; V79 cells	no tested	500 - 2000 µg/ml	negative	no information on toxicity purity: substance was purchased from Hoechst	Sala et al., 1982

Table 4.15 In vitro tests: Chromosomal aberrations

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			
chromosomal aberrations; CHO cells	160 - 1600 µg/ml	160 - 1600 µg/ml	negative	treatment / sampling time: 2/14 h (with S-9 mix) and 12/14 h (without S-9 mix) no information on toxicity purity: 98 %	Galloway et al., 1987 (also citated in NTP, 1991)

Table 4.16 In vitro tests: sister chromatid exchanges (SCE)

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			

V79 cells	490 - 700 µg/ml	343 - 3000 µg/ml	positive	marginally positive with and without S-9 mix: 1. <u>experiment/without S-9 mix</u> dose (µg/ml) SCE/cell neg.co. 5.6 343 6.8 490 7.0 700 **8.2** 1000 **7.4** <u>1. experiment/with S-9 mix</u> dose (µg/ml) SCE/cell neg.co. 7.1 490 8.5 700 9.7 2. <u>experiment/without S-9 mix</u> dose (µg/ml) SCE/cell neg.co. 4.6 2000 7.0 3000 9.0 with and without S-9 mix no positive controls without S-9 mix strongly toxic at the highest tested dose purity: substance was purchased from Hoechst	Sala et al., 1982

CHO cells	160 - 1600 µg/ml	5.0 - 160 µg/ml	equivocal	slight increase of SCE in one out of two experiments with S-9 mix; negative without S-9 mix 1. experiment/with S-9 mix dose (µg/ml) SCE/cell neg.co. 8.3 160 9.2 500 10.1 1600 9.9 po.co. 22.6 2. experiment/with S-9 mix dose (µg/ml) SCE/cell neg.co. 9.2 1200 9.3 1400 10.1 1600 10.6 po.co. 21.2 no informations on toxicity purity: substance was purchased from NTP chemical repository	Galloway et al., 1987 (also cited in NTP, 1991)

Table 4.17 In vitro tests: unscheduled DNA syntheses (UDS)

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			
human WI-38 cells	0.0005 - 0.5 µl/ml (0.5 - 500 µg/ml)	0.001 - 0.1 µl/ml (1.0 - 100 µg/ml)	negative	liquid scintillation counting toxicity: no data purity: no data	Stauffer Chemical Company, 1979

Table 4.18 In vivo tests: Rodent bone marrow tests with mice

Test system	Doses (mg/kg bw)	Expos. regimen	Sampl. Times	Result	Local cytotox.	General toxicity	Remarks	Reference
CD-1 mice; bone marrow erythrocytes	175 - 700	1 x i.p.	16, 24 and 48 h	negative	decre-ased P/N ratio at highest tested dose at 48 h - sampling	lethal effects at the highest tested dose clinical signs from 350 mg/kg upwards	highest tested dose corresponds to the maximum tolerated dose 5 males and 5 females per group purity: 99.2 %	IRI, 1993
NMRI mice; bone marrow erythrocytes	1000	1 x p.o.	24, 48 and 72 h	negative	no data	toxic signs lethal effects (3/30)	tested dose corresponds to the maximum tolerated dose 5 males and 5 females per group purity: 99.0 %	Otto, 1984
Chinese hamsters; bone marrow erythrocytes	62.5 - 250	1 x i.p.	24 h	equivocal	no data	no data	although a slight increase of micronucleated cells was observed the overall result was characterized as questionable by the authors dose MN cells (%) neg. co. 0.35 62.5 0.52 125 0.66 250 0.70 po.co. 7.06 2 males and 2 females per group purity: no data	Sala et al., 1982

Table 4.19 In vivo tests: Tests with Drosophila melanogaster

Test system	Exposure	Result	Remarks	Reference
mitotic recombination	feeding: 2.5 - 40 mmol/l were dropped on the surface of the food	negative	purity - the only information: substance was commercially available	Vogel and Nirvard, 1991

4.1.2.8 Carcinogenicity

Carcinogenicity studies by gavage conducted to modern regulatory standards are available in rats and mice. These studies provide clear evidence that tris(2-chloroethyl)phosphate is carcinogenic in two animal species. Tris(2-chloroethyl)phosphate causes benign and malignant tumors at various organ sites in rats and mice.

4.1.2.8.1 Studies in animals

In vivo studies

- ***Oral***

Gavage studies (rat and mouse)

Rats

Groups of 60 male and 60 female F344/N rats were administered by gavage to tris(2-chloroethyl)phosphate in corn oil for 5 days per week for up to 103 weeks (NTP 1991; Matthews 1993). Ten animals per sex per group from each species were designed for interim evaluation (necropsy, hematology, and clinical chemistry) at 66 weeks.

Rats of each sex received 0, 44, or 88 mg/kg bw /d tris(2-chloroethyl)phosphate (purity 98%). Results of non-neoplastic findings at interim sacrifice (66 weeks) and at 103 weeks were described in detail in section 4.1.2.6.
The survival of female rats receiving 88 mg/kg bw /d and, to a lesser extent, male rats was reduced in relation to controls. The reduced survival of females was attributed, in part, to the neurotoxicity of tris(2-chloroethyl)phosphate and to a marginally increased incidence of mononuclear cell leukemia. Final mean body weights of surviving rats were similar to those of controls. The principal tris(2-chloroethyl)phosphate-related effects occurred in the kidney and brain of dosed rats (for brain lesions see section 4.1.2.6).Oral administration of tris(2-chloroethyl)phosphate to rats for up to two years was associated with a marked increase in the incidence of neoplastic lesions in the kidney, mainly proliferative lesions and adenomas of the renal tubule. They occurred with a dose-related increased incidence in both males and females. Hyperplasia and adenomas occurred in nearly 50% of the 88 mg-dose males and in 10% of the 44 mg-dose males. In male rats receiving 88 mg/kg bw tris(2-chloroethyl)phosphate renal tubule hyperplasia were seen in: control 0/50; low-dose 2/50; high-dose 24/50; renal tubule adenoma in: control 1/50; low-dose 5/50; high-dose 24/50 and, to a lesser extent, in female rats renal tubule hyperplasia was observed in: control 0/50; low-dose 3/50; high-dose 16/50, and renal tubule adenoma: control 0/50; low-dose 2/50; high-dose 5/50. The increased incidences in the 88 and 44 mg/kg bw/d groups for adenomas in the

kidneys exceeded the NTP historical control range for this lesion. The historical incidence of renal tubule adenoma of the kidney in control gavage (corn oil) F344/N rats from previous 2-year studies up to 1989 is 0.2% (0-2% range) in males and 0.1% (0-2% range) in females (NTP 1991; Matthews 1993). The same range for the overall mean historical incidences of renal tubule adenoma of the kidneys in vehicle control F344/N rats from recent 2-year carcinogenicity studies by the NTP was reported in another review (Eustis 1994). There was also less than 1% [0.75% (0-2% range) in males and 0.19% (0-2% range) in females]. The renal tubule proliferating lesions were small. The historical incidence of hyperplasia in the kidney in control gavage F344/N rats is 1.18% (0-6% range) in males and 0.10% (0-2% range) in females (Eustis 1994). Renal tubule carcinoma occurred in one control and one high-dose male rat. The overall rate of carcinomas in the NTP historical control gavage F344/N rats was also very small, 4/1,069 (0.37%) for males and 0/1,068 (0.00%) for females (Eustis 1994). Thus, the renal response in rats appears to be restricted to hyperplasia and benign tumors. Adenoma and carcinoma of the renal tubule constitute a morphological continuum, and there are no cytologic features that clearly and unambiguously distinguish the more benign neoplasms from those with the ability to metastasize. It is assumed that the renal adenomas represent an early stage of the development of a carcinoma. Therefore, the marked increase in the incidence of renal tubule neoplasms in F344/N rats gavaged with tris(2-chloroethyl)phosphate is considered to represent reasonable clear evidence of carcinogenic activity.
Thyroid follicular cell neoplasms in male and female rats may have been related to tris(2-chloroethyl)phosphate administration. The incidence of thyroid gland follicular cell neoplasms (adenomas) was slightly increased (male rats: control 1/50; low-dose 2/48; high-dose 3/50; female rats: control 0/50; low-dose 1/50; high-dose 1/50). Thyroid follicular cell neoplasms occurred with a significant positive trend in female rats, and the incidence of follicular cell adenoma or carcinoma combined was significantly greater in the 88 mg/kg bw/d females than in controls. The incidence of follicular cell neoplasms was also increased in the 88 mg/kg bw/d males, but this increase was not statistically significant. The combined incidence of follicular cell neoplasms in the 88 mg/kg bw/d male and female rats equals or exceeds the upper rates for NTP historical controls from previous 2-year studies up to 1989 (males: 51/2,106, 2.4, range 0-10%; females: 34/2,107, 1.6%, range 0-6%). However, the low incidence of follicular cell hyperplasia did not support an effect of tris(2-chloroethyl)phosphate on the thyroid gland; no hyperplasia was seen in males, and only one dosed and one control female had follicular cell hyperplasia. The lack of hyperplasia in rats argues against considering the follicular cell neoplasms as related to tris(2-chloroethyl)phosphate since most thyroid carcinogens also induce hyperplasia. Therefore, it is uncertain whether the thyroid follicular cell neoplasms in the F344/N rats are related to the administration of tris(2-chloroethyl)phosphate.

Mononuclear cell leukemia in male and female rats may have been related to tris(2-chloroethyl)phosphate administration. The incidence of mononuclear cell leukemia was increased in both dosed male and female rats (male rats: control 5/50; low-dose 14/50; high-dose 13/50 and female rats: control 14/50; low-dose 16/50; high-dose 20/50). There were significant positive trends in both sexes, and the incidence in males receiving 44 or 88 mg/kg bw/d and females receiving 88 mg/kg bw/d was significantly greater than in their respective controls, but it is uncertain whether these were related to tris(2-chloroethyl)phosphate administration. Since the frequency of mononuclear cell leukemia in dosed male and female rats was within in the range of historical controls from previous 2-year studies up to 1989 (males:2-44%, females: 0-33%, s. key at the end of the Table 4.19). Thus, for both males and females the highest rate is within the historical control range. Therefore, these marginal

increases in leukemia in male and female F344/N rats were not considered to be clearly related to administration of tris(2-chloroethyl)phosphate. In the brain, benign granular cell tumors were observed in 3/50 males at 88 mg/kg bw/d only. There were no treatment-related increases in the incidence of tumors in the brain of females. The incidence of the mostly observed neoplastic lesions in F344/N rats in the 2-year gavage study of tris(2-chloroethyl)phosphate was given in the following table, Table 4.20.

Table 4.20

Summary table: Incidence of neoplastic lesions in F344/N rats after life-time exposure to tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

Doses	0	44 mg/kg bw/d	88 mg/kg bw/d
Male			
Kidney: Renal tubule			
Hyperplasia, overall rates[a]	0/50 (0%)	2/50 (4%)	24/50 (48%)
Historical control data[b]:	12/1,019 (1.18%); range 0%-6%		
Adenoma, overall rates[a]	1/50 (2%)	5/50 (10%)	24/50 (48%)
Carcinoma, overall rates[a]	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adenoma or Carcinoma, overall rates	2/50 (4%)	5/50 (10%)	25/50 (50%)
Historical control data[b]:	12/2,142 (0.6% ± 0.9%); range 0%-2%		
Thyroid Gland: Follicular Cell			
Hyperplasia, overall rates[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adenoma, overall rates[a]	1/50 (2%)	2/48 (4%)	3/50 (6%)
Carcinoma, overall rates[a]	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adenoma or Carcinoma, overall rates	1/50 (2%)	2/50 (4%)	5/50 (10%)
Historical control data[c]:	51/2,106 (2.4% ± 2.3%); range 0%-10%		
Hematopoietic System: Mononuclear Cell Leukemia			
Overall rates[a]	5/50 (10%)	14/50 (28%)	13/50 (26%)
Historical control data[d]:	321/2,149 (14.9% ± 10.8%); range 0%-44%		
Female			
Kidney: Renal tubule			
Hyperplasia, overall rates[a]	0/50 (0%)	3/50 (6%)	16/50 (32%)
Historical control data[b]:	1/1,019 (0.10%); range 0%-2%		
Adenoma, overall rates[a]	0/50 (0%)	2/50 (4%)	5/50 (10%)
Historical control data[e]:	1/2,144 (0.1% ± 0.3%); range 0%-2%		
Thyroid Gland: Follicular Cell			
Hyperplasia, overall rates[a]	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adenoma, overall rates[a]	0/50 (0%)	1/50 (2%)	1/50 (2%)
Carcinoma, overall rates[a]	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adenoma or Carcinoma, overall rates[a]	0/50 (0%)	3/50 (6%)	4/50 (8%)
Historical control data[f]:	341/2,107 (1.6% ± 1.6%); range 0%-6%		
Hematopoietic System: Mononuclear Cell Leukemia			
Overall rates[a]	14/50 (28%)	16/50 (32%)	20/50 (40%)

Historical control data[g]:	329/2,150 (15.3% ± 10.6%); range 0%-33%

[a] Number of tumor-bearing animals/number of animals examined at site; [b] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [c] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [d] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [e] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [f] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [g] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation)

Overall, in a standard carcinogenicity study (2-year gavage study) in F344/N rats, there was clear evidence of carcinogenic activity for males and females receiving tris(2-chloroethyl)phosphate as shown by increased incidences of renal tubule adenomas at ≥44 mg/kg bw /d (below the maximum tolerated dose level, MTD), and statistically significantly high incidences at 88 mg/kg bw/d. Moreover, focal renal tubule hyperplasia, a possible precursor of adenoma, was also seen in about half the 88 mg/kg bw/d males.

Mouse

The 2-year carcinogenicity study in B6C3F1 mice was conducted by administering (gavage) 0, 175, or 350 mg/kg bw tris(2-chloroethyl)phosphate (purity 98%) in corn oil to groups of 60 male and 60 female mice, 5 days per week for up to 103 weeks (NTP 1991, Matthews 1993). Ten animals per sex per group from each species were designed for interim evaluation (necropsy, hematology, and clinical chemistry) at 66 weeks. Results of non-neoplastic findings at interim sacrifice (66 weeks) and at 103 weeks were described in detail in section 4.1.2.6.

There were no significant differences in survival between dosed and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal tris(2-chloroethyl)phosphate-related effects occurred in the kidney. In the original diagnosis, in which single sections of left (through the longitudinal plane) and right (through the transverse plane) kidneys were examined microscopically, the following neoplastic lesions were observed: tubule cell adenomas in one control male, one low-dose female, and one high-dose male, and an adenocarcinoma was seen in another high-dose male. Karyomegaly (nuclear enlargements) in the proximal convoluted tubules was noted in the kidneys of appr. 80% of mice receiving 350 mg/kg bw/d and less frequently in mice receiving 175 mg/kg bw/d. Focal hyperplasia, a potentially preneoplastic lesion, was seen in a high-dose male. Thus, it has to operate on the assumption that long-life administration of tris(2-chloroethyl)phosphate may lead to marginal increase in renal tubule neoplasms, and increased incidences of renal tubule hyperplasia also in mice. However, the interpretation of animal carcinogenicity tests traditionally rely almost exclusively upon a comparison of specific tumor rates in treated vs. matched and, perhaps, historical control animals. Another approach to aid interpretation of the data was to examine multiple sections of the kidney. Because of the occurrence of tris(2-chloroethyl)phosphate-related kidney neoplasms in F344/N rats, the association of karyomegaly with kidney neoplasms known from other chemicals, and the low spontaneous incidence of kidney neoplasms in historical control, additional sections of the kidney were prepared from each control and treated mice, examined microscopically, and evaluated to provide a clearer indication of the potential effect of tris(2-chloroethyl)phosphate in this

organ and to give more data for comparison of the dosed and control groups, and therefore, to allow a more rigorous statistical comparison with concurrent controls. In this subsequent examination of step-sections of all the mouse kidneys, two additional adenomas were observed in high-dose males and one in a low-dose female. Focal hyperplasia was observed in one additional control male and four additional high-dose males (two from the 66-week interim and two from the 2-year group).

A survey of the incidence of selected renal tubule cell lesions and neoplastic findings in the Harderian gland in B6C3F1 mice in the 2-year gavage study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993) was given in the following table, Table 4.21.

Table 4.21

Summary table: Incidence of selected renal tubule cell lesions and neoplastic findings in the Harderian gland in B6C3F1 mice after life-time exposure to tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

Doses	0	175 mg/kg bw/d	350 mg/kg bw/d
Male			
Kidney: Renal tubule			
Karyomegaly, overall rates[a]	2/50 (4%)	16/50 (32%)	39/50 (78%)
Hyperplasia, original and step sections combined[a]	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adenoma, original and step sections combined[a]	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adenocarcinoma, original and step sections combined[a]	0/50 (0%)	0/50 (0%)	1/50 (2%)
Historical control data[c]:	5/2,183 (0.2%), 0-2% range		
Historical control data[d]:	4/949 (0.42%), 0-2% range)		
Female			
Kidney: Renal tubule			
Karyomegaly, overall rates[a]	0/50 (0%)	5/49 (10%)	44/50 (88%)
Hyperplasia, original and step sections combined[a]	0/50 (0%)	1/49 (2%)	2/50 (4%)
Adenoma, original and step sections combined[a, e]	0/50 (0%)	1/49 (2%)	0/50 (0%)

Historical control data[d]:	0/949 (0.00%)		
Harderian Gland			
Hyperplasia, combined[a, b]	1/58 (2%)	4/59 (7%)	2/59 (3%)
Adenoma, combined[a, b]	3/59 (5%)	7/60 (12%)	9/60 (15%)
Adenoma or carcinoma, combined[a, b]	3/59 (5%)	8/60 (13%)	10/60 (17%)
Historical control data[e]:	53/2,193 (2.4%), 0-10% range		
Historical control data[f]:	18/609 (3.0%)		
Historical control data[g]:	(2.8%)		
Historical control data[h]:	(5.1%)		

[a] Number of tumor-bearing animals/number of animals examined at site; [b] combined data from the 66-week interim evaluation and 104-week terminal evaluation; [c] 2-year historical incidence of renal tubule adenomas for vehicle control groups in NTP corn oil gavage studies up to 1989 (NTP 1991); [d] 2-year historical incidence of renal tubule adenomas for vehicle control groups data, analyzed from 16 recent NTP studies (Eustis et al. 1994); [e] 2-year historical incidence of Harderian gland tumors for vehicle control groups in NTP corn oil gavage studies up to 1989 (NTP 1991); [f] Morphology of spontaneous Harderian gland tumors in aged B6C3F1 mice (Ihara M et al., 1994); [g] Spontaneous tumors in aging (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice (Tamano et al., 1988); [h]Frequency and time of spontaneous tumors found in B6C3F1 mice oncogenicity studies over 10 years (Eiben R, 2001)

In male B6C3F1 mice a further evaluation of renal tumors using the results of original single sections combined with those of step-sections revealed the following incidences of renal tubule neoplasms 1/50 (2%), 1/50 (2%), and 4/50 (8%) for males. In female mice, during the initial examination of single sections, one adenoma was observed in the low-dose group. The step-sections did not identify any additional neoplasm in females, although it did identify hyperplasia in one low- and two high-dose female mice. In male mice, the observed incidences for adenoma in the kidneys in the 350 mg/kg bw/d group exceeded the NTP historical control range for this lesion. Renal tubule neoplasms have been observed infrequently in gavage vehicle (corn oil) control B6C3F1 mice. The historical incidence of renal tubule adenoma of the kidney in control gavage (corn oil) male B6C3F1 mice from previous 2-year studies up to 1989 reported in the presented NTP study is 0.2% (5/2,183; 0-2% range)(NTP 1991). In another report examined 16 recent NTP studies the historical incidences of renal tubule adenoma of the kidney in control gavage (corn oil) B6C3F1 mice were 4/949 (0.42%; 0-2% range) in males and 0/943 (0.00%) in females (Eustis et al. 1994). Although the slight increase in tubule cell neoplasms in male B6C3F1 mice was not statistically significant, the marginal increase in both tubule cell hyperplasia and tubule cell neoplasms is suggestive of a substance-related effect. The 8% incidence of renal tubule neoplasms in the 350 mg/kg bw/d males is greater than the overall rate of 0.2-0.42% for the groups of historical control gavage male mice for which multiple sections were evaluated. In addition to these observations, the incidences of renal tubule hyperplasia and the rates of atypical renal tubule epithelial cells were also increased. The nucleoli were increased in size and number or were too large for size of the cell (increased nuclear: cytoplasmic ratio). Dose-related increased rates of karyomegaly in cells of renal tubule epithelium were noted in both male and female mice given ≥175 mg/kg bw/d when compared to controls. Based on the additional results received from the resection examination and the observations of increased renal proliferative lesions and also of cell atypia in renal tubule epithelium in mice treated

with tris(2-chloroethyl)phosphate, and because renal tubule neoplasms are uncommon in control B6C3F1 mice, thus, the data in the kidneys were considered to provide a clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male B6C3F1 mice.

Furthermore, there was equivocal evidence of carcinogenic activity for female B6C3F1 mice as shown by a marginally increased incidence of Harderian gland adenomas. In females receiving 350 mg/kg bw/d tris(2-chloroethyl)phosphate the incidence of Harderian gland neoplasms, primarily adenomas, was marginally increased relative to concurrent controls. There were adenoma or carcinoma: control 3/50, low-dose 8/50, and high-dose 7/50; in addition, three Harderian gland neoplasms occurred in the 350 mg-dose female mice evaluated after 66 weeks. If the incidence rates for the interim sacrifice groups and 2-year animals are combined, there is a significant trend, and the incidence in the 350 mg/kg bw/d group is significantly greater than that in controls (adenoma or carcinoma: 3/59, 8/60, 10/60). Few reports are available regarding the incidence of spontaneous Harderian gland tumors in mice. However, the frequency in all strains is low. The historical incidence of Harderian gland tumors in control gavage (corn oil) female B6C3F1 mice from previous 2-year studies up to 1989 reported in the presented NTP study is 2.4% (53/2,193; 0-10% range) (NTP 1991). In another survive spontaneous Harderian gland tumors in B6C3F1 mice were found in 18 (3.0%) of 609 females (Ihara et al. 1994), and in 2.8% females sacrificed in studies between weeks 79 to 104 (Tamano et al., 1988). In a further study evaluating frequency and time of spontaneous tumors in B6C3F1 mice in oncogenicity studies over 10 years, Harderian gland adenomas were found in 5.1% females (Eiben 2001). In this view the incidences of Harderian gland tumors in both dose groups of females exceeded the range of the concurrent controls and also of the historical controls. There were no corresponding tris(2-chloroethyl)phosphate-related increase incidence of hyperplasia of the Harderian gland in female mice. Therefore, this marginal increase was considered as equivocal evidence of carcinogenic activity to tris(2-chloroethyl)phosphate.

Overall, in a standard carcinogenicity study (2-year gavage study) in B6C3F1 mice, some evidence of carcinogenic activity for male mice was obtained by a marginally increased incidence of renal tubule cell neoplasms at 350 mg/kg bw/d tris(2-chloroethyl)phosphate, and for females as shown by a markedly increased incidence of Harderian gland adenomas at the lowest tested dose of 175 mg/kg bw/d tris(2-chloroethyl)phosphate and above.

Diet study (mouse)

Groups of 50 male and 50 female Scl:ddY mice were fed a commercial diet containing approximately 0, 0.012, 0.06, 0.3, and 1.5% (equivalent to 0, 12, 60, 300, and 1500 mg/kg bw/d, calculated on an assumed body weight of 20 g and food consumption of 10% body weight/day) tris(2-chloroethyl)phosphate (purity 98%) over a period of 18 months (Takada et al., 1989). This study differs in some respects from the published guideline OECD TG 451. There are basic data comparable to this guideline with respectable restrictions. The test parameter documented do not totally comply with this testing guideline for carcinogenicity, however, there was a well-documented study reports which meets basic scientific standards. The supplied data are considered as valid for use in risk assessment.

In both sexes fed 1500 mg/kg bw/d tris(2-chloroethyl)phosphate, mortality rates were clearly higher (approximately 40% survival compared to around 65% survival in controls) and body

weight gain was apparently suppressed compared to other groups (approximately 60% lower than the control value). Survival of groups dosed ≤300 mg/kg bw/d was not significantly different than that of controls, and no symptoms of toxicity were observed in mice of either sex. No differences in food consumption were found between the groups for both sexes. In hematology, no changes were found apart from significant elevation in platelets in males of the 300 mg/kg bw/d dose group. Significant declines were found in the weights of the heart and testes in males and kidneys in females from the 1500 mg/kg bw/d dose group. At histopathological examinations, hyperplasia, and hypertrophy of the urinary tubule epithelium together with karyomegaly were noted in the kidney of all treated animals. These nuclei were polymorphic, and several times abnormal division, degeneration and necrosis were observed. Unfortunately, no incidences of these findings were reported. In addition, cysts of the kidneys, necrosis and interstitial fibrosis were observed only in animals of the 1500 mg/kg bw/d dose group. No such findings in the kidney were reported in controls.

Increased tumor incidence was observed in the kidney when compared to concurrent controls. The renal tumors included renal cell adenomas with proliferation of tumor cells into tubules, cell heteromorphy and poor cell division, and renal cell carcinomas in which the tumor cells reproduced substantially, were polymorphic and showed cell division. An increased incidence of renal adenomas was reported in males at 300 mg/kg bw/d and above, and in females at the top dose. The following incidences of adenomas were observed: in males (0/50, 0/49, 0/49, 2/47, 9/50), and in females (0/49, 0/49, 0/50, 0/49, 2/50). Furthermore there was an increased incidence of renal carcinomas in males (2/20, 0/49, 2/49, 3/47, 32/50) and females (1/50 at 1500 mg/kg bw/d and zero in all other groups). Although the number of kidney tumors observed in male mice at dose of 300 mg/kg bw/d was small compared to concurrent controls, this finding was assessed as relevant, especially in terms of spontaneous renal neoplasms in mice occur infrequently. When they do develop, the affected mice are generally at least at the age of 12 months and are usually older than 18 months (Cohen and Friedell, 1982). Spontaneous occurring renal adenomas and carcinomas are rare in all mice strains with exception of strain BALB/cf/Cd and various lines of Swiss mice (CD-1, CF-1, Swiss-Webster, Swiss-E1). Males seem to be more frequently affected than females. The following frequencies of spontaneous epithelial tumors of male mouse kidney cortex are reported: B6C3F1: 0.1%, 3/2543 (Ward et al., 1979); C57BL/6CrSlc: 0.4%, 2/463 (Nakamura et al., 1992); SWR/J: 0.1%, 1/671 (Rabstein et al., 1973); CD1 Ha M/ICR: 0.2%, 2/1000 (Percy and Jonas, 1971); SPF Swiss Webster derived: 1.9%, 2/101 (Sher, 1974); CF-1 Swiss: 3/55, 5.4% (Tomatis et al., 1972); BALB/cf/Cd: 60-70%, 215-251/358 (Rabstein and Peters, 1973). It is even more rare in females, with reported incidences in B6C3F1: 0.08%, 2/2522 (Ward et al., 1979); C57BL/6CrSlc: 0.2%, 1/463 (Nakamura et al., 1992); SWR/J: 0 (Rabstein et al., 1973); CD1 Ha M/ICR: 0 (Percy and Jonas, 1971); SPF Swiss Webster derived: 0 (Sher, 1974); CF-1 Swiss: 1/56, 1.8% (Tomatis et al., 1972); BALB/c: 0.5%, 4/791 (Frith, 1986); BALB/cf/Cd: 40%, - (Claude, 1958). Unfortunately, there are no published historical control data for the ScI:ddYY mouse strain available. However, there are data from concurrent control groups of a 2-year study investigating the chronic toxicity and the carcinogenicity of 1,2,4-trichlorbenzene on the skin using the ScI:ddYY mouse strain (Yamamoto, 1982). In the control group of 50 male and 50 female mice skin-painting was carried out with acetone alone. The incidence of kidney tumors was zero in animals of both sexes. Further data from concurrent controls were found in a study which examined the induction of renal and liver tumors in male ddY mice by Ochratoxin A, a mycotoxin (Kanisawa and Shigetoshi, 1978). In the control group of 10 male mice fed basal died for 50 weeks no renal cell tumors and no atypical tubule cells were observed. The data presented showed that the very low incidence of neoplasms in the kidneys of the concurrent control groups of ScI:ddYY mice in the described

study and the zero incidence of the concurrent control groups in the 2-year study of 1,2,4-trichlorobenzene corresponds with the low frequency for spontaneous kidney tumors in several mouse strains reported in the literature.

Increased tumor incidences were also observed in the liver of male mice when compared to concurrent controls. There were adenomas in which the tumor cells proliferated and compressed the surrounding liver cells. They showed enlarged cytoplasm and poor cell division, together with carcinomas in which the tumor cells were heteromorphic with atypical-sized or contained multiple nuclei presenting a funicular structure with two or more cell layers. The incidence of benign hepatocellular adenomas was significantly increased in the 300 mg/kg bw/d male dose group and above. In addition there was a slight , but not dose-related, increase in the incidence of liver carcinomas in males (1/50, 1/49, 4/49, 2/47, 3/50). The liver is the most common target site in the mouse, and among mutagens as well as nonmutagens. Hepatocellular adenoma and hepatocellular carcinoma occur spontaneously in many strains. The incidence of spontaneous liver tumors in mice varies greatly among strains. In common, in a given strain, spontaneous hepatocellular neoplasms are much more frequent in males than in females. The following frequencies of spontaneous liver tumors were reported: B6C3F1: 7-58% in males, 0-21% in females (Tarone et al., 1980); C57BL/6CrSlc: 9.5% in males, 1.8% in females (Nakamura et al., 1992); CF-1: 21-39% in males (Turusov et al., 1973). In the above cited 2-year study of 1,2,4-trichlorobenzene on the skin using the ScI:ddYY mouse strain no liver tumors were noted in male and female mice of the concurrent control groups (Yamamoto, 1982). In the other long-term study of ochratoxin A using male ddY mice hyperplastic nodules in the liver were found in two of 10 male control mice fed only basal diet for 50 weeks (Kanisawa and Shigetoshi, 1978). Thus, there was stated that the liver tumor incidences for the concurrent controls reported in the described study with ScI:ddYY mice confirmed these published data. In view of this results, it was concluded that tris(2-chloroethyl)phosphate is an oncogenic substance which may induce tumors in the liver of +ScI:ddYY mice.

In females, significant increases were found in the incidence of papillomas and squamous cell carcinomas (combined) in the forestomach of the 1500 mg/kg bw/d dose group, and in those of leukemia of 300 mg/kg bw/d and 1500 mg/kg bw/d dose groups. However, the relevance of these findings in the forestomach for human health risk assessment is questionable, since humans have no forestomach. However, rodent forestomach carcinogens may promote human carcinogenesis in other, different anatomical sites. In addition, some other tumors, like lung adenomas and mammary carcinomas, and leukemia in females, occurred in greater number in the treated groups but with no significant difference in incidence between tris(2-chloroethyl)phosphate treated groups and controls. A survey of tumor incidences in the kidneys and liver in Scl:ddY mice exposed to tris(2-chloroethyl)phosphate via the diet over a period of 18 months was given in the following table, Table 4.22.

Table 4.22

Summary table: Incidence of renal cell tumors, and hepatocellular tumors seen in Scl:ddY male mice after an 18 months exposure period via the diet to tris(2-chloroethyl)phosphate (Takada et al., 1989)

Doses (mg/kg bw/d)	0	12	60	300	1500
Male					
Kidney					
Adenoma, overall rates[a]	0/50 (0%)	0/49 (0%)	0/49 (0%)	2/47 (4.3%)	9/50 (18%)
Carcinoma, overall rates[a]	2/50 (4.1%)	0/49 (0%)	2/49 (4.1%)	3/47 (6.4%)	32/50 (64%)
Adenoma and Carcinoma, combined[a]	2/50 (4%)	0/49 (0%)	2/49 (4.1%)	5/47 (10.6%)	41/50 (82%)
Liver					
Adenoma, overall rates[a]	3/50 (6%)	4/49 (8.2%)	3/49 (6.1%)	10/47 (21.3%)	16/50 (32%)
Carcinoma, overall rates[a]	1/50 (2%)	1/49 (2%)	4/49 (8.2%)	2/47 (4.3%)	3/50 (6%)
Adenoma and Carcinoma, combined[a]	4/50 (8%)	5/49 (10.3%)	7/49 (14.3%)	12/47 (25.5%)	19/38 (82%)

[a] Number of tumor-bearing animals/number of animals examined at site

Overall, tris(2-chloroethyl)phosphate was clearly carcinogenic following oral administration in male Scl:ddY mice. There were increased tumor incidences in the kidney and liver when compared to the concurrent controls. Clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male Scl:ddY mice was observed by a dose-related increased incidence of tumors in the kidneys at ≥300 mg/kg bw/d (statistically significantly high incidences of tumors at 1500 mg/kg bw/d); and by an increased incidence of tumors in the liver at ≥60 mg/kg bw/d (statistically significantly high incidences of tumors at ≥300 mg/kg bw/d).

In summary, the data of the highest dose level of 1500 mg/kg bw/d were excluded from further viewing because the top dose exceeded the MTD (maximum tolerated dose) for this mouse strain shown by reduced survival and reduction in body weight gain (>10%). However, based on the results presented in the employed dose range it was concluded that increased incidence of kidney and liver tumors in Scl:ddY mice occurred at ≥300 mg/kg bw/d in the diet for 18 months. At this dose range increased tumor rates were also determined in the study with B6C3F1. In addition, the tumor incidences of the concurrent controls reported in this mouse study confirmed the published data for spontaneous kidney and liver tumors of other mouse strains. From the data presented it appears that Scl:ddY mice were not particularly more susceptible to renal cell and liver tumors than other mouse strains tested. In consideration of the concurrent controls and control data obtained with the same mouse strain in two other bioassays it is quite evident that the incidences of kidney tumors and hepatocellular tumors of animals of the 300 mg/kg bw/d dose group lie above the frame of the historical data. The data in the kidneys and liver were considered to provide a clear evidence of tris(2-chloroethyl)phosphate induced carcinogenic activity in male Scl:ddY mice. Because of increased rates of renal proliferative lesions and of cell atypia in renal tubule epithelium which were observed in animals treated at ≥12 mg/kg bw/d, a NOAEL for kidney tumor formation could not established. However, 60 mg/kg bw/d is considered to be the NOAEL for liver tumor formation in this study.

- ***Dermal***

Mice

Groups of 25 Swiss mice at 45 days of age were used in an *in vivo* short-term skin test for studying the sebaceous gland suppression and for induction of epidermal hyperplasia. Animals received dorsal applications on days 1, 3 and 5 of tris(2-chloroethyl)phosphate (purity unspecified) at 0, 31.9, 53.2, 74.5 mg in an acetone solution (total dose applied in three applications). Benzo(a)pyrene was used as positive control. The treated skin areas were removed on day 8 and the number of sebaceous glands and the thickness of epidermis were measured by standard procedures. No suppression of the sebaceous glands and no hypoplasia were induced in the skin of mice treated with tris(2-chloroethyl)phosphate. However, data are without relevance within the overall database for the test substance (Sala et al., 1982).

In a short reported study, groups of 32 female mice received skin applications of tris(2-chloroethyl)phosphate (purity unspecified), each of 21 mg per day, in acetone, 2 times/week on shaved skin for 78 weeks. No skin tumors were obtained. One hepatoma and one endometrial sarcoma were found, and an increased incidence of lung adenomas (12/32) was reported. Thus, the test produced no convincing evidence of carcinogenic potential for tris(2-chloroethyl)phosphate by the dermal route. Due to limitations of experimental design (lack of controls) and reporting, no firm conclusion can be made (Sala et al., 1982).

Tumor promotion

In addition, tris(2-chloroethyl)phosphate was tested for initiating/promoting activity in Swiss mice by skin application (Sala et al., 1982). Groups of 35 female Swiss mice, nine weeks of age, received skin applications of tris(2-chloroethyl)phosphate (purity unspecified) in acetone to examine its initiating potential. One group of mice received a single application of 71 mg/mouse tris(2-chloroethyl)phosphate followed by applications of 1 µg/mouse tetradecanoyl phorbol acetate (TPA) twice a week for 78 weeks. A control group was treated with TPA alone. The animals were examined regularly and the time of appearance of papillomas and of malignant neoplasms on skin were recorded. Systematic post-mortem and histological examinations were performed on all animals. The incidence of squamous-cell papillomas of the skin was 17/33 (52%) tris(2-chloroethyl)phosphate plus TPA-treated animals and 12/28 (48%) TPA-treated mice; squamous-cell carcinomas of the skin developed in 2/33 tris(2-chloroethyl)phosphate plus TPA-treated animals but none of the TPA-treated controls. The incidences of lung adenomas were 7/33 and 5/28 in tris(2-chloroethyl)phosphate plus TPA-treated and in TPA-treated groups, respectively. Tris(2-chloroethyl)phosphate showed no significant complete carcinogenic, initiating or promoting activity on mouse skin and lung. Nevertheless, the promoting activity and complete carcinogenicity of tris(2-chloroethyl)phosphate in this study could not be evaluated because of the lack of negative controls.

Overall, there are no dermal carcinogenicity studies according to modern test design to tris(2-chloroethyl)phosphate. In the skin test for sebaceous gland suppression and for the induction of epidermal hyperplasia, there was neither a decrease in the number of sebaceous glands nor an increase in the thickness of the epidermis. The findings of the initiation/promotion studies with tris(2-chloroethyl)phosphate showed no significant carcinogenic, promoting or initiating potential on mouse skin and lung. But limitations in study design and reporting do not allow firm conclusions to be drawn.

- ***Inhalation***

No information available.

Summary of animal in vivo data on carcinogenicity to tris(2-chloroethyl)phosphate

There is sufficient evidence for the carcinogenicity of tris(2-chloroethyl)phosphate in experimental animals. There are positive results in two animal species. There were similarities in the target organs. Evidence of carcinogenicity in the kidneys has been observed in both rats and mice. Carcinogenic potential in rats and mice was demonstrated for the oral route. There are standard carcinogenicity oral (gavage) studies using F344/N rats and B6C3F1 mice (NTP 1991, Matthews 1993). There was clear evidence of carcinogenic activity in male and female F344/N rats. In the F344/N rat, tris(2-chloroethyl)phosphate caused an increased incidence of renal tubule adenomas in both males and females after oral administration of 44 mg/kg bw/d and above for up to two years. These adenomas occurred in nearly 50% of male F344/N rats receiving 88 mg/kg bw/d and in 10% of the F344/N males receiving 44 mg/kg bw/d (below the maximum tolerated dose level, MTD). To a lesser extent a statistically significant increase was observed in female F344/N rats (88 mg/kg bw/d: 10%, 44 mg/kg bw/d: 4%). In view of the results obtained in a similar study with B6C3F1 mice it seemed mice are less sensitive than rats to the effects of tris(2-chloroethyl)phosphate, because doses in the carcinogenicity study were about four times greater than those administered to F344/N rats. In the one carcinogenicity study in B6C3F1 mice, evidence of carcinogenic activity for male B6C3F1 mice was obtained by a marginally increased incidence of renal tubule cell neoplasms at 350 mg/kg bw/d (below the MTD). The evidence for carcinogenicity was shown by the increased incidence of tubule cell neoplasms (2 additional adenomas and two hyperplasias in 350 mg/kg bw/d dose male mice) obtained in supplemental evaluation of additional kidney sections. There was an increased incidence of tubule cell neoplasms of 8%, which was outside the overall rates of 0.2-0.42% for the groups of control gavage male mice and the historical control range of 0-2% (NTP 1991). Although the slight increase in tubule cell neoplasms was not statistically significant, the markedly increase in both tubule cell hyperplasia together with cell atypia in renal tubule epithelium in both male and female mice given ≥175 mg/kg bw/d and tubule cell neoplasms are suggestive of a treatment-related effect and are considered to present evidence of carcinogenic activity for male B6C3F1 mice. For female B6C3F1 mice there was an equivocal evidence of carcinogenic activity as shown by a marginal increased incidence of Harderian gland adenomas at the lowest tested doses of 175 mg/kg bw/d and above. The incidences of Harderian gland tumors in both dose groups were greater than those in the concurrent controls and also in historical controls (2.4% NTP 1991; 3.0%, Ihara et al., 1994, 2.8%, Tamano et al., 1988, and 5.1%, Eiben, 2001).

A further carcinogenicity study revealed significant increases in the incidence of tumors in the kidney and liver of male Scl:ddY mice after administration of tris(2-chloroethyl)phosphate in the diet for 18 months. However, this study differs in some respect from published guidelines, but is comparable to OECD TG 451 with acceptable restrictions. Clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male Scl:ddY mice was observed by a dose-related increased incidence of tumors of the kidneys at ≥300 mg/kg bw/d and above; and by an increased incidence of tumors in the livers at ≥60 mg/kg bw/d (statistically significantly high incidences of tumors at 300 mg/kg bw/d (below the MTD), which were each outside the range of concurrent controls and the historical range of other mouse strains (Takada et al., 1989).

There is no report available on the carcinogenicity of tris(2-chloroethyl)phosphate after inhalation.

There are no dermal carcinogenicity studies according to guideline study (OECD, etc.) to tris(2-chloroethyl)phosphate available. The findings that are available are negative, but limitations in study design and reporting do not allow firm conclusions to be drawn.

In vitro studies

Cell transformation assays

There are two cell transformation tests to tris(2-chloroethyl)phosphate available.

In a test using SHE cells (without S-9 mix) an increase in the transformation rate was observed, however, without dose-response relationship. Increases over control of 3.3% (1.4 transformed colonies/plate) at 400 µg/ml tris(2-chloroethyl)phosphate and of 1.2% (0.6 transformed colonies/plate) at 500 µg/ml tris(2-chloroethyl)phosphate were measured. At 600 µg/ml tris(2-chloroethyl)phosphate a negative response was observed. Toxic effects were induced at 800 µg/ml (Sala et al., 1982).

Another cell transformation assay (type III foci) using C3H10T1/2 cells was negative with and without S-9 mix up to 1500 µg/ml tris(2-chloroethyl)phosphate. However, the methodology of the test and the results are insufficiently documented (Sala et al., 1982).

Summary on cell transformation tests:

Results of the two cell transformation tests showed controversial findings of tris(2-chloroethyl)phosphate. In the test system with SHE cells (without S-9 mix) an increased transformation rate was noted, however, negative data were described in another test using C3H10T1/2 cells with and without S-9 mix. Nevertheless, these very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity.

Concern from mutagenicity data:

The conclusion is that there is no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate as bacterial gene mutation tests were negative, *in vitro* genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, and two valid *in vivo* mammalian micronucleus tests and a Drosophila test were negative.

Discussion on carcinogenicity

There is no indication to assume that the tumors induced in rats and mice may be related to primary genotoxic effects. The existence of other/alternative (non genotoxic) mechanisms is assumed.

Kidney tumors
Evidence in the rat: *F344/N rats (male and female)*
Evidence in the mouse: *B6C3F1 mice (male), Scl:ddY mice (male)*

In rodent bioassays, the kidney is one of the most frequent sites for the induction of cancer by chemicals, contrasting with the situation in humans where the kidney is an infrequent site of primary cancer development. The main portion of these studies shows positive evidence for male rat kidney tumors; renal tumor induction is a much less frequent response in mice. Whereas, results of the 2-year rat study showed evidence of kidney tumors not only in male F344/N rats but also in females, and in addition, treatment-related kidney tumors were also observed in males of two mouse strains, after treatment for two years in B6C3F1 mice and after 18 months in Scl:ddY mice.

Not all rodent renal carcinogens (or their reactive metabolites) are genotoxic, acting by way of direct adduct formation with kidney DNA. There are also examples of secondary mechanisms of chemical carcinogenesis in rat kidney. It is known that tubules of the renal cortex are particularly vulnerable because this region received about 80% of the renal blood flow. In addition, the proximal tubule cells of the kidney have a high capacity for transporting organic ions and certain other substrates directly from the peritubular circulation into the tubular fluid and vice versa. This process can lead to significant levels of nephrotoxicants within the tubule cell. Next to the liver, the kidney is critical site for biotransformation of many different chemicals, being endowed with enzymatic pathways for carrying out extensive oxidative, reductive, hydrolytic, and conjugative processes. Information from metabolic studies on rats and mice give clues to conditions of action and possible mechanism of action to tris(2-chloroethyl)phosphate showing the kidney as target organ. So, it is assumed that the kidneys were preferentially exposed to large quantities of tris(2-chloroethyl)phosphate distributed by the circulatory system. Most chemicals with tumorgenic activity in rodent kidney require metabolic activation to a reactive species termed the ultimate carcinogen. The knowledge of the biochemical and molecular mechanisms of renal carcinogenesis in rodents is far from complete. The kidney possesses a number of enzymatic functions, which not only represent detoxification or synthetic mechanisms, but are also capable of metabolic activation of chemicals. One of the metabolic pathways involved in the proximal tubules is the cytochrome P450 monooxygenase system.

It has been argued that sustained tissue damage and subsequent cell proliferation is a common predisposing factor for cancer development in rodents, that is dependent on a specific metabolic process, e.g. halogenated alkenes. These chemicals are biotransformed to nephrotoxic metabolites via a multistep pathway involving the cytosolic enzyme β-lyase. The selective renal action of these compounds commences with glutathione S-conjugate formation in the liver, transport of the S-conjugate to the kidney where it is metabolized to a cysteine S-conjugate by one of several brush border enzymes, mainly γ-glutamyltranspeptidase, and finally renal bioactivation by cysteine conjugate β-lyase to yield cytotoxic or mutagenic metabolites. The constant stimulus of cytotoxicity leads to compensatory tissue repair and cell proliferation which in time ends up in the formation of benign renal tumors.

Although there may be interspecies differences at the level of specific enzymes, these various predisposing factors apply equally to humans and to rodents. However, one physiological peculiarity that would predispose the male rat kidney, but not the human kidney, to the carcinogenic action of certain chemicals, is the unique presence in male rats of high urinary levels of a specific protein, namely $\alpha_{2\mu}$-globulin. This protein belongs to the lipocailin family, is synthesized in high levels in the male liver, and is involved in an independent secondary tumor induction mechanism in the rat kidney identified in various chemicals that can induce $\alpha_{2\mu}$-globulin nephropathy. These renal carcinogens include a diverse group of chemicals, mainly light hydrocarbons used as fuels and the food constituent *d*-limonene, that are known

to induce a renal syndrome termed hyaline droplet nephropathy involving accumulation of male rat-specific urinary protein, $\alpha_{2\mu}$-globulin. The induction of renal tubule tumors by this diverse group of chemicals only in male rats correlates with the absence of high circulating levels of $\alpha_{2\mu}$-globulin in female rats and the complete absence of $\alpha_{2\mu}$-globulin in male and female mice. In this context, there are no indications of renal tumor development caused by tris(2-chloroethyl)phosphate interactions with $\alpha_{2\mu}$-globulin.

Another factor that may predispose the male rat to renal tumor formation is the spontaneous age-related condition encountered in virtually all carcinogenicity bioassays, chronic progessive nephropathy (CPN). CPN is a degenerative condition which affects male rats more severely than females. There is also a sustained regenerative component to CPN because many affected tubules are characterized by a marked increase in proliferative activity, and atypical tubule hyperplasia (a preneoplastic lesion) has been reported to occur in very advanced stages of the disease, e.g. hydroquinone.

In general, humans and rodents develop a similar spectrum of tumor types in the kidney. The main renal tumor types in rats and mice were renal tubule adenoma and carcinoma. Tubule tumors are also the most important kidney tumors in humans. In the kidneys of tris(2-chloroethyl)phosphate exposed F344/N rats and both B6C3F1 and Scl:ddY mice increased tumor rates of the same anatomical site were observed. There were primarily renal tubule adenoma, but carcinoma occurred also. Nevertheless, the mechanism of tumor development in the kidneys of tris(2-chloroethyl)phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that tris(2-chloroethyl)phosphate appears to have no genotoxic potential. A skin-painting carcinogenicity study with one of the possible toxic metabolite 2-chloroethanol failed to demonstrate carcinogenicity. However, renal carcinogens acting through those causing secondary or indirect DNA effects can be assumed to represent equivalent risks for humans. There are no data on the mechanism(s) of toxicity and carcinogenicity action of tris(2-chloroethyl)phosphate. It is likely that carcinogenicity would be mediated by non-genotoxic (epigenetic) mechanisms. However, a species-specific mechanism of tumor formation in the kidney was not identified. Perhaps the major factor underlying the renal carcinogenesis is the rate of blood flow through the kidney. This combined with a reactively high metabolic capability suggests a possible step of action in tumor response to tris(2-chloroethyl)-phosphate. There is postulated that tris(2-chloroethyl)-phosphate may have induced renal tumors in the F344/N rat secondary via cytotoxicity followed by cell proliferation mechanism e.g. hydroquinone, halogenated alkenes. But, there are data suggestive of evidence of carcinogenicity also in female F344/N rats and in male mice of two mouse strains following chronic exposure to tris(2-chloroethyl)-phosphate. So, the mechanism of tumor formation following tris(2-chloroethyl)-phosphate exposure is not definitively established.

Renal carcinogens acting via a secondary or indirect mechanism involve threshold phenomena for their tumor-inducing or nephrotoxic effects. However, the problem is that there are possibly many different modes of action thought to be involved in the renal tumor formation in tris(2-chloroethyl)-phosphate administered rats and mice. To date, there is a uncertainty identification of the crucial event, especially on the primary toxic effects of concern for tris(2-chloroethyl)-phosphate following development of tumors in animal cancer bioassays. However, the difficulty is that the determination of a threshold dose for non-genotoxic carcinogens is based by fully knowledge of the cascade of effects following interaction of the substance with its biological target(s). For this reason, at present it may be extremely difficult or impossible to identify a threshold for the underlying toxicity of kidney

tumor development to tris(2-chloroethyl)-phosphate. In F344/N rats tumor formation was observed in animals of both sexes at all dose tested, ≥44 mg/kg bw/d. Based on this data, the NOAEL for kidney tumor formation could not established for male and female F344/N rats (NTP 1991; Matthews 1993). An increased kidney tumor rate and increased incidences of renal tubule hyperplasia were noted in male B6C3F1 mice after treatment with the high dose of 350 mg/kg bw/d for two years whereas increased rates of cellular atypia of renal tubule epithelial cells such as karyomegaly were seen in both male and female mice given ≥175 mg/kg bw/d (NTP 1991, Matthews 1993). At 300 mg/kg bw/d, which presented the same dose range seen adenomas in the kidneys in male B6C3F1 mice, an increased incidence of renal tubule adenomas were noted in male Scl:ddY mice after treatment with tris(2-chloroethyl)-phosphate in the diet for 18 months. However, findings in the kidneys observed as hyperplasia and hypertrophy of the urinary tubule epithelium together with nuclei enlargement were determined in male Scl:ddY mice fed at ≥12 mg/kg bw/d when compared to controls (Takada et al., 1989). Therefore, a NOAEL for tumor formation in the kidney could not be established. Overall, no conclusion can be drawn about the relevance of these tumors to humans due to the lack of knowledge of the possible mode of action. However, considering all available data, the relevance to humans is probably very significant.

Liver tumors
Evidence in the mouse: Scl:ddY mice (male)

Due to its anatomical position and its enzymatic content, the liver is the organ most frequently affected in toxicological studies on rodents and is also the organ most frequently affected in carcinogenicity studies in rats and mice. The mechanism of tumor development in the liver of tris(2-chloroethyl)phosphate treated Scl:ddY mice was not studied.

Non-genotoxic mouse liver carcinogens are known to have different mode of action. In the literature a link between persistent liver damage and the development of liver tumors was discussed for non-genotoxic carcinogens. This phenomenon is not unique to the liver since necrosis and regeneration are associated with a high incidence of tumors in other organs, notably urothelium (Grasso 1987a, 1987b). Special tests examining the effect of subcellular injury on the turnover rate of liver cells were not done in the presented mouse study.

In addition, there are also some non-genotoxic compounds inducing liver enlargement and mitogenesis without unequivocal signs of hepatotoxicity. There was discussed that the early phases of hepatomegaly are associated with mitogenic effects that can be measured as cell in S-phase within the first few days of administration. The later stages of hepatomegaly appear to be associated more with cellular hypertrophy. Further, sustained hepatomegaly induced by chemicals is associated with proliferation of the smooth endoplasmatic reticulum (SER), peroxisomes and the induction of a range of liver enzymes. There were no results available that such mode of action or potential interaction of modes of action may operate in tris(2-chloroethyl)phosphate treated mice.

In summary, at present there is no clear data on the mechanism of liver carcinogenesis by tris(2-chloroethyl)phosphate in the male Scl:ddY mice. However, it is conceivable that liver cell damage and feature of the hepatic reparative response may be responsible for tumor production to tris(2-chloroethyl)phosphate. In the study with Scl:ddY mice local necrosis, and vacuolation of liver cells were observed in all treatment groups (≥12 mg/kg bw/d) but including the controls whereas no such findings were reported from the other mouse study.

Based on the results presented there was stated that tris(2-chloroethyl)phosphate is an oncogenic substance which may induce tumors in the liver of male ScI:ddYY mice. The dose level of 60 mg/kg bw/d is considered to be the NOAEL for tumor formation in the liver. Overall, no conclusion can be drawn about the relevance of this tumors to humans due to the lack of knowledge of the possible mode of action. However, considering all available data, the relevance to humans is probably significant.

Harderian gland tumors
Evidence in the mouse: B6C3F1 mice (female)

The Harderian gland is one of the few organs that is unique to a limited number of animal species; for example, primates do not have Harderian glands. Harderian gland neoplasms are not an unusual finding in mice; the incidence of this tumor, as with most other neoplasms of mice, increases with age. Because of the post-orbital location of the gland, large tumors can cause exophthalmos. In the 2-year study on B6C3F1 mice, an equivocal evidence of carcinogenic activity as shown by a marginal increased incidence of Harderian gland adenomas in females was noted at the lowest tested doses of 175 mg/kg bw/d tris(2-chloroethyl)phosphate and above. However, the actual incidence of neoplasms of this gland in this study was under-reported because microscopy was performed only on those glands which had visible gross lesions (NTP 1991, Matthews 1993). In conclusion, the incidences of Harderian gland tumors in both dose groups of females were greater than those in the concurrent controls and also in historical controls (2.4%, NTP 1991; 3.0%, Ihara et al., 1994, 2.8% Tamano et al., 1988, and 5.1% Eiben, 2001).

Due to the fact that the Harderian gland has no human counterpart and in absence further relevant information of the possible mode of action these tumors are considered of no relevance to humans.

4.1.2.8.2 Human data

No data are available.

4.1.2.8.3 Summary and conclusion on carcinogenicity

From animal data it is obvious that there is a cancerogenic potential of tris(2-chloroethyl)phosphate. There are relevant guideline cancer studies using F344/N rats and B6C3F1 mice available (NTP 1991, Matthews 1993). In addition data of a diet study for 18 months using Scl:ddY mice comparable to guideline study with acceptable restrictions is available (Takada et al., 1989).

Carcinogenic potential of tris(2-chloroethyl)phosphate in rats and mice was demonstrated for the oral route.

Tris(2-chloroethyl)phosphate caused primarily benign tumors but also and malignant tumors in the kidney in F344/N rats (males and females) and also in male mice of two mouse strains (B6C3F1, Scl:ddY). Rats and mice of both strains developed a similar spectrum of tumor

types in the kidneys. Additionally tumor development after tris(2-chloroethyl)phosphate treatment was seen in the liver of male in Scl:ddY mice, and in Harderian gland of B6C3F1 female mice, respectively. The data in the kidneys were considered to provide a clear evidence of tris(2-chloroethyl)phosphate induced carcinogenic activity in male Scl:ddY mice. Because of increased rates of renal proliferative lesions and of cell atypia in renal tubule epithelium which were observed in animals treated at ≥12 mg/kg bw/d, a NOAEL for kidney tumor formation could not established. Thus, for risk characterisation purposes a LOAEL of 12 mg/kg bw/d is brought forward for tumor formation.

An increased incidence of renal tubular cell adenomas and carcinomas was observed in male and female F344/N rats at ≥44 mg/kg bw/d (below MTD); statistically significantly high incidences of tumors at 88 mg/kg bw/d (NTP 1991, Matthews 1993). In male B6C3F1 mice increased incidences of renal tubule cell neoplasms and of renal tubule cell hyperplasia were reported at 350 mg/kg bw/d (below MTD). In addition, increased rates of cellular atypia of renal tubule epithelium cells such as karyomegaly were noted in both male and female mice given ≥175 mg/kg bw/d (NTP 1991, Matthews 1993). Dose-related increased incidence of tumors in the kidneys were also seen in male Scl:ddY mice fed diet concentrations of 300 mg/kg bw/d (below MTD) and above for 18 months. Also in male Scl:ddY mice statistically significantly high incidences of tumors (adenomas and carcinomas) in the liver were noted at 300 mg/kg bw/d (below MTD) and above. In female B6C3F1 mice marginally increased incidence of Harderian gland adenomas was seen at the lowest tested doses of 175 mg/kg bw/d and above (NTP 1991, Matthews 1993).

No species-specific mode of action for tris(2-chloroethyl)phosphate carcinogenesis was identified.

A reasonable threshold mechanism could not be identified for all tumors and tumor sites. Cytotoxicity was assumed as underlying mode of carcinogenesis in the kidney although indications on cytotoxicity, inflammation or the involvement of apoptosis are presently absent or not available. However, signs of increased proliferation and karyomegaly of renal tubule epithelial cells were seen in F344 rats of both sexes at ≥44 mg/kg bw/d and in male B6C3F1 mice at ≥175 mg/kg bw/d (NTP 1991, Matthews 1993) as hyperplasia and hypertrophy of the urinary tubule epithelium together with nuclei enlargement in male Scl:ddY mice fed at ≥12 mg/kg bw/d for 18 months (Takada et al., 1989). No other non-genotoxic mode of action was identified.

Persistent cell damage and development of tumors was also discussed for non-genotoxic modes in the liver. In the study with Scl:ddY mice local necrosis, and vacuolation of liver cells were observed in all treatment groups (≥12 mg/kg bw/d). However, other non-genotoxic modes might be suspected, but not yet verified. A NOAEL for the cytotoxic effects was not established, and also not for cell proliferation mechanism.

The carcinogenic effect of tris(2-chloroethyl)phosphate is thought to be related to non-genotoxic (epigenetic) mechanisms.

According to the decision of the EU C&L WG tris(2-chloroethyl)phosphate will be classified as a carcinogen, category 3 and labelled as Harmful, Xn, R 40.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Fertility impairment

Animal studies

Tris(2-chloroethyl)-phosphate (purity ≥ 98 %) was investigated for possible impairment of reproduction and fertility in CD-1 mice in a study using the reproductive assessment continuous breeding protocol (RACB) by oral administration (gavage) at dose levels of 175, 350 and 700 mg/kg bw/d (Gulati et al., 1991). The dose levels used during this study had been obtained from a preceding 14-day treatment dose finding study. At the beginning of the continuous breeding phase, groups of 40 pairs were used for the control (corn oil) and of 20 pairs for the treated groups. Animals were treated for a total of 18 weeks (1 week before mating, 14 weeks held as breeding pairs and three weeks thereafter). The last litters born during the holding period following the continuous breeding phase were reared by their dams until weaning, after which treatment of these F1 animals was initiated by the same route and at the same concentration as F0 parental animals. At the age of 74 ± 10 days 20 non-sibling F1 animals per treatment group were mated for 1 week and held until delivery. In addition, a separate one-week crossover mating trial had been performed to determine the affected sex. This mating trial consisted of three groups of 20 pairs each: control males x control females, control males x 700 mg/kg bw/d treated females, 700 mg/kg bw/d treated males x control females. Endpoints evaluated during the continuous breeding study were clinical signs of toxicity, parental body weight, parental average water consumption during representative weeks, fertility (number of pairs producing a litter/number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex of live pups and the pup body weight immediately after birth. At necropsy of the animals the livers, kidneys, testes, epididymides, prostate, seminal vesicles with coagulating glands and ovaries were weighed and fixed for histopathological evaluation. The endpoints evaluated for the F1 animals and for those of the crossover mating trial were the same as for the continuous breeding part of the study. In addition, for these two latter parts of the study also epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity were evaluated.

F0 animals (continuous breeding phase):

No treatment related mortalities were observed and no clinical signs were reported. Water consumption in the treated groups was similar to that of the controls. Mean body weight of the animals (combined males and females) was similar in comparison to the controls during the mating and holding period. All pairs were fertile in terms of delivering at least one litter. However, while there was no difference between the 175 mg/kg dose group and the control in the number of pairs producing five consecutive litters (17/19 versus 35/38 in the controls) during the continuous breeding phase, the number of pairs producing a 5^{th} litter was statistically significantly reduced in the 350 mg/kg dose group (13/18 versus 35/38 in the controls). This effect was even more pronounced in the high dose treated groups. No 4^{th} or 5^{th} litter at all was produced in the 700 mg/kg dose group, only 2 out of 18 pairs delivered a 3^{rd} litter and also the number of pairs producing a 2^{nd} litter was statistically significantly reduced (12/18 versus 37/38 in the controls). TCEP treatment also revealed a dose related decrease in the average numbers of live pups/litter which was statistically significantly reduced at the 350

mg/kg dose group for the 3rd (9.3 ± 0.9 pups/litter versus 13.1 ± 0.7 pups/litter in the control), 4th and 5th litter and at the 700 mg/kg dose group for the 2nd (4.3 ± 1.1 pups/litter versus 13.1 ± 0.6 pups/litter in the control) and 3rd litter. Absolute pup birth weights were increased in the mid and high dose groups, however, the differences were not statistically significant when adjusted for litter size. The cumulative days-to-litter values were significantly greater for the second litter in the 700 mg/kg/d dose group (65.9 ± 6.4 days) in comparison to the control group (40.8 ± 0.3 days).

F1 generation (last litters from continuous breeding phase):

Since poor fertility and pup survival in the 700 mg/kg/d group left insufficient animals for the second generation only the 175 and 350 mg/kg/day treated dose groups were performed. No treatment related effects were seen for the body weights of both male and female parental animals and for their water consumption data. Percentage of pregnant (90%) and fertile (90%) pairs in the 175 mg/kg dose group were comparable to that of the controls (85%, resp. 89%). In the 350 mg/kg dose group percentages of pregnant (70%) and fertile (70%) pairs were decreased, but ratios were not statistically significantly different from the controls. The average number of live pups/litter was statistically significantly reduced in the 350 mg/kg dose group (7.6 ± 1.1 pups/litter versus 11.4 ± 0.5 pups/litter in the control) and less male pups were found in both of the treated groups when compared to the controls.

Cross over mating trial:

Pregnancy and fertility indices were statistically significantly lower in the high dose treated male/control female group in comparison to both the control male/control female group or the control male/high dose treated female group. Only one out of 18 pairs of the high dose treated male/control female group produced a litter. Average number of live pups/litter were statistically significantly lower in both of the treated groups (from treated males: 3.0 pups/litter; from treated females: 7.2 ± 0.9 pups/litter) in comparison to the control (10.3 ± 0.7 pups/litter).

Necropsy results on organ evaluation:

At daily doses of 700 mg/kg bw the males of the cross over mating trial revealed statistically significantly higher absolute and relative liver weights, statistically significantly lower absolute and relative kidney/adrenal and right testis weights and statistically significantly lower absolute right epididymides weights. At daily doses of 350 mg/kg bw the parental F1 males showed statistically significantly lower absolute and relative right epididymides weights. At 175 mg/kg/d no differences were observed in the parental F1 males for right caudal, right epididymal and right testes weights in comparison to the controls. At daily doses of 700 mg/kg bw the females of the cross over mating trial revealed statistically significantly lower absolute and relative kidney/adrenal weights. At daily doses of 350 mg/kg bw the parental F1 females showed statistically significantly increased absolute kidney/adrenal weights. No effects on the evaluated organ weights were observed at the 175 mg/kg/d dose level.

Sperm evaluations:

At daily doses of 700 mg/kg bw evaluated from the cross over mating trial epididymal sperm density per gramme caudal tissue was statistically significantly reduced to about 810 x 10^6 in comparison to controls (about 1223 x 10^6), percentage epididymal sperm motility was

statistically significantly reduced to about 35% in comparison to the controls (about 78%) and percentage abnormal sperm was statistically significantly increased to about 32 % (controls: 9 %). Also, testicular spermatid head count data revealed statistically significant reduction in average spermatid count and total spermatid heads per gramme testis tissue. At daily doses of 350 and 175 mg/kg bw evaluated in the parental F1 animals no differences were observed for percentage epididymal sperm motility, epididymal sperm density and percentage abnormal sperm in comparison to the control groups

Vaginal cytology evaluations:

Neither for the 700 mg/kg/d dose level evaluated in females during the cross over mating trial nor for the 350 and 175 mg/kg/d dose level evaluated in the F1 females any effects on average estrous cycle length or estrual cyclicity had been revealed.

In a further study (Gulati and Russel, 1985, Morrissey et al. 1988) tris(2-chloroethyl)-phosphate was evaluated for effects on sperm parameters and on vaginal cytology (SMVC evaluations) in F-344 rats and in B6C3F1 mice. This study is indicated to have been part of NTP-sponsored 13-week subchronic tests in rodents. Groups of ten male and female animals were treated by gavage with doses of 44, 175, and 700 mg/kg/d (mice) and with 22, 88, and 175 mg/kg/d (rats) for approximately 18 weeks. Endpoints determined for the males at necropsy included right testis weight, right epididymal weight, right caudal weight, sperm motility, sperm number per gramme caudal tissue and sperm morphology, and for the females estrual cyclicity . In male B6C3F1 mice tris(2-chloroethyl)-phosphate administered at doses of 44 and 175 mg/kg bw/d had no significant effect on whole body weight, right caudal, right epididymal, or right testis weights and on sperm motility, sperm count, or on the incidence of abnormal sperm in comparison to controls. At 700 mg/kg bw/d a significant decrease was noted with respect to right epididymal and right testicular weights. Furthermore, at this dose level the average sperm count per gramme caudal tissue was statistically significantly decreased and the incidence of abnormal sperm was statistically significantly elevated relative to the control values. In male F-344 rats tris(2-chloroethyl)-phosphate administered at doses of up to and including 175 mg/kg bw/d had no significant effect on whole body weight, right caudal, right epididymal, or right testis weights, or on the incidence of abnormal sperm. At 175 mg/kg bw/d sperm count was significantly higher in comparison to the controls, whereas percent sperm motility as slightly decreased (77% versus control value of 89%). In female F-344 rats tris(2-chloroethyl)-phosphate treatment had no apparent effect on estrual cyclicity. In female B6C3F1 mice an increased length of the estrous cycle and variations in the relative frequencies of estrous stages were found for the 44 and 175 mg/kg bw/d groups, however, not at the high dose group.

Three studies with repeat administration of TCEP (Stauffer 1980a, Takada et al., 1989 and NTP 1991) gave some further hint that the male reproductive system might be a target (cf. chapter 4.1.2.6, Table 4.11).

4.1.2.9.2 Developmental toxicity

Animal studies

Teratogenic effects of tris-(2-chloroethyl)phosphate were investigated in fetuses and offspring of Wistar rats (Kawashima et al., 1983, summary available only). The substance (suspended in olive oil) was given by gavage to pregnant dams day during days 7 to 15 of gestation. Each

group consisted of 23-30 animals and received doses of 50, 100, and 200 mg/kg bw/d. No changes in maternal body weight gain, food consumption and general appearance were found in the groups of 50 and 100 mg/kg. At the highest dose level of 200 mg/kg 7 out of 30 dams died. Also maternal food consumption was markedly depressed and clinical symptoms such as piloerection and general weakness were recorded. It is reported that on the 20th day of gestation there was no increase in embryonic/fetal death and/or gross malformations attributable to treatment with tris(2-chloroethyl)phosphate at any dose level examined. There was a small increase in skeletal variations (cervical ribs, varied sternebrae, lumbar ribs) at the two higher dose levels. In the postnatal examination, the development of the offspring in all groups examined was well maintained without any disorders attributable to the treatment in morphological examination and in some functional tests such as open field, water maze, rota rod, slop test, pain reflex and Peyer´s reflex examinations. The authors concluded that tris(2-chloroethyl)phosphate had been revealed not to be teratogenic in rats even at maternally toxic doses.

With regard to the developmental study of Kawashima et al. (1983) death of 7 dams out of 30 was reported to have occurred during gestational days 10 to 14. For the latter ones, slight piloerection and weakness and explicitely no signs of intoxication were reported. Animals had not been necropsied or further investigated. Maternal death, however, was obviously of no impact, since development of the conceptus as well as postnatal development of the offspring of the 23 dams that were carried on from the 200 mg/kg dose group remained unaffected.

Tris(2-chloroethyl)phosphate was also included in the evaluation of a total of 60 chemicals within a Chernoff-Kavlock screening assay on CD-1 mice (Hardin et al., 1987). Dams had been treated by gavage during g.d. 6 to 13 with 940 mg/kg bw/d. Maternal weight gain was significantly less in treated dams in comparison to controls. However, there were no effects on the number of viable litters, live born/litter, percentage survival, birth weight and weight gain of the pubs.

Human data

No data available.

Other information

From a very poorly documented study (Shepel´skaja and Dyschinewitsch, 1981) it is reported, that male rats (strain and group size not specified, details of the test protocol not provided) were kept in inhalation chambers with a volume of 200 l and during a course of 4 months were exposed to TCEP concentrations (no further data on chemical identity/purity or vapour generation provided) of 0.5 or 1.5 mg/m^3. During the course of the study, males were mated (no further information provided) to check reproductive function, and pregnant females investigated on day 21 of gestation. As a result, it is reported that spermatology on males from the 1.5 mg/m^3 exposure group revealed no change in the number of sperm, however a diminution in the number of actively motile sperm, yet with unimpaired duration of motility. No changes were reported for the 0.5 mg/m^3 exposure group. Indication on some abnormal sperm morphology were reported, however sperm head morphology was unaffected. Histology of the testes reported a slight increase in the spermatogenesis index in both

exposure groups, increases in number of spermatogonia in seminiferous tubules, increased incidences of desquamated epithelial cells in the tubular interstitium and a decrease in number of tubules with cells in meiotic stages. In females mated to males from the 0.5 or 1.5 mg/m^3 exposure group an increase in pre- and postimplantation embryonic death and a lower number of progeny was reported. In females mated to males from the 0.5 mg/m^3 exposure group an increase in postimplantation embryonic death was reported as well as lower weight and size of the foetuses in comparison to controls. Due to very poor documentation and reporting the study is not considered sufficient for the purpose of risk assessment.

4.1.2.9.3 Summary and conclusion on reproductive toxicity

Tris(2-chloroethyl)phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice. Reproductive failure was observed at daily doses of 700 mg/kg bw with at best and no more than 3 litters produced and with no pups surviving from the last litter produced. The findings were essentially confirmed from the results of a separate cross over mating trial in mice at the same dose level. The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl)phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains. Based on a statistically significant reduction of the number of litters produced by the F0 generation, reduced pregnancy and fertility indices in the F1 generation, and statistically significantly reduced litter size in both the F0 and the F1 generations a NOAEL/fertility of 175 mg/kg bw/d was derived from the study in CD-1 mice with oral administration (Gulati and Chapin, 1991).

A firm conclusion on developmental toxicity is hampered by poor reporting of rather old data as only a summary report and a reporting from a screening assay are available. However, it appears, that tris(2-chloroethyl)phosphate has no embryo-/fetotoxic or specific teratogenic properties in mice and rats even at maternally toxic doses. A NOAEL/developmental toxicity of 200 mg/kg bw/d and a NOAEL/maternal toxicity of 100 mg/kg bw/d was derived from a study with rats with oral administration (Kawashima et al., 1983).

Based on the available animal data tris(2-chloroethyl)phosphate is identified as a reproductive toxicant with a significant toxic potential adverse to fertility. Treatment of mice resulted in significant impairment of reproductive success of both sexes and of male reproductive organs and of sperm parameters. Therefore, tris(2-chloroethyl)phosphate will be classified and labeled as reproductive toxicant Cat. 2, R60. No significant toxicity to embryo-/fetal development has been revealed from tris(2-chloroethyl)phosphate treatment of pregnant rats.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Data on human experience with tris(2-chloroethyl) phosphate (TCEP) are not available. TCEP is well absorbed (> 90% of the dose) and distributed in rats after oral administration.

Higher concentrations were found in liver and kidney up to 24h after administration. An enterohepatic circulation is supposed to occur. Metabolism and elimination are the same after single and repeated application. Metabolites in urine were identical in rats and mice. Main metabolites were bis(2-chloroethyl) carboxymethylphosphate, bis(2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl)-2-hydroxyethyl-phosphate glucuronide. Data are not available for inhalation and dermal exposure. For risk characterisation purposes, the rates of oral, dermal, and inhalation absorption are assumed to be 100%.

TCEP demonstrated moderate toxicity after oral application (LD50 for rats in the range of 430-1230 mg/kg bw). In an experiment with rabbits, the substance has demonstrated low acute dermal toxicity (LD50 > 2150 mg/kg bw)**.** In experiments with rats low acute inhalation toxicity was detected.

Draize tests with rabbits revealed weak irritation of the skin and mild irritation of the conjunctivae. TCEP is not considered to be a skin and eye irritant.

Human data on skin sensitisation potential of the substance are not available. An animal study (Buehler Test) showed no skin sensitizing potential of TCEP. Sensitisation data of the two other chloroalkyl phosphates TCPP and TDCP which are structurally related to TCEP indicate that these substances do not possess significant skin sensitisation potential. Taking into consideration all information on the three chloroalkyl phosphates TCEP, TCPP and TDCP including alkylating properties of these substances it is concluded that TCEP should be non-sensitizing to humans.

No information is available on the respiratory sensitisation potential of TCEP and the two other chloroalkyl phosphates.

Kidneys, brain and liver appeared to be the main sites of the toxic attack in experimental animals after repeated oral application of tris(2-chloroethyl) phosphate (dose ranges from 22 to 700 mg/kg bw/d in rats and up to 1500 mg/kg bw/d in mice).

Kidneys appear to be the most sensitive organ for repeated exposure of tris(2-chloroethyl) phosphate. Kidney effects observed in rats and mice were dose- and time-related with respect to incidence and severity. Kidney findings were noted in Sprague-Dawley and F344/N rats and in B6C3F1 and Scl:ddY mice. In both rat strains and both mouse strains kidney lesions of the same kind were observed. In subchronic toxicity studies a NOAEL for kidney lesions was estimated (for male and female Sprague-Dawley rats at 3000 ppm, 192 and 215 mg/kg bw/d, resp., and for B6C3F1 mice at 350 mg/kg bw/d. (Stauffer Chemical Company 1980b). However, a NOAEL for kidney effects could not be derived for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d tris(2-chloroethyl) phosphate, furthermore, for B6C3F1 mice after long-life exposure to ≥175 mg/kg bw/d (NTP 1991), and for Scl:ddY mice after feeding of ≥12 mg/kg bw/d in the diet for 18 months (Takada et al. 1989). 12 mg/kg bw/d is considered as LOAEL for kidney lesions and will be used for risk characterisation.

Brain effects following repeated oral application of TCEP were found in rats, but not in mice. A dose- and sex-dependent neuronal necrosis in the hippocampal and thalamal region of the brain was observed. This lesion is more common and more severe in female than in male rats. In addition, gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of some female rats were found. Degenerative lesions in the brain occurred in rats in a dose related pattern, and in addition, in a clear time-response relationship

in frequency, intensity and severity. Male rats were less sensitive to adverse brain effects than female rats. The lowest NOAELs for systemic brain effects were derived in each case from subchronic and chronic oral toxicity studies for rats at dose levels between 44 to 175 mg/kg bw/d. With respect to brain effects mice were less sensitive to TCEP than rats. The NOAEL for brain effects was established to be 44 mg/kg bw/d in F344 rats.

Liver effects observed in F344/N rats and B6C3F1 mice after treatment with TCEP were limited to increased organ weights without other corroborating findings. The increase in liver weights was not considered to be toxicologically adverse.

No data on local effects after repeated exposure to TCEP are available.

Serum cholinesterase activity was inhibited in female F344/N rats receiving 175 and 350 mg/kg bw/d tris(2-chloroethyl) phosphate by gavage for 16 weeks, but not in male F344/N rats. There were no such tris(2-chloroethyl) phosphate-related differences in cholinesterase activity in B6C3F1 mice of both sexes in subacute as well as subchronic studies up to 700 mg/kg bw/d. The inhibition of serum cholinesterase activity can only be considered as an indication for exposure to TCEP, but not for prediction of ill-health effects.

Although several chloroalkyl phosphates have shown to produce delayed neurotoxicity in hens, a very high oral dose of tris(2-chloroethyl) phosphate (14200 mg/kg bw) did not cause behavioral effects or nerve damage suggestive of neurotoxicity in White Leghorn hens. Clinical signs of neurotoxicity, including ataxia, excessive salivation, gasping, and convulsion were noted in female F344/N rats receiving double the target levels of 175 and 350 mg/kg bw/d TCEP for 3 days in week four during an oral 16-week gavage study. In addition, female rats dosed with 175 and 350 mg/kg bw/d TCEP experienced occasional periods of hyperactivity after dosing. Male and female B6C3F1 mice given 350 and 700 mg/kg bw/d TCEP for 16 days exhibited ataxia and convulsive movements during the first 3 days of dosing.

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, in a mouse-lymphoma-assay and in a UDS-test. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Two in vivo mice micronucleus tests were negative for application up to maximum tolerated doses, while a positive result of questionable validity was observed in another test. Also a Drosophila test was negative. Overall, it can be concluded that there is no relevant evidence for mutagenicity of tris(2-chloroethyl) phosphate.

There are no human data on carcinogenicity. Tris(2-chloroethyl) phosphate is carcinogenic in both sexes of rats and mice. It causes the formation of predominantly benign tumors in the kidney, but also benign and malignant tumors at various sites in experimental animals. It induces kidney tumors in F344/N rats at doses ≥44 mg/kg bw/d, in male B6C3F1 mice at 350 mg/kg bw/d; and in male Scl:ddY mice at diet concentrations of 300 mg/kg bw/d and above. In addition, dose-related increased incidences of hyperplasia and hypertrophy of the urinary tubule epithelium together with karyomegaly were also observed in these animals. Such findings were noted in male and female F344/N rats at doses ≥44 mg/kg bw/d, in both male and female B6C3F1 mice given ≥175 mg/kg bw/d, and in male Scl:ddY mice fed at ≥12 mg/kg bw/d tris(2-chloroethyl) phosphate. The value of 12 mg/kg bw/d is considered as LOAEL for tumor formation. In addition, TCEP induces tumors in the liver of male Scl:ddY mice at 300 mg/kg bw/d and above, and in the Harderian gland of female B6C3F1 mice at

≥175 mg/kg bw/d. Since there is no evidence of a direct genotoxic mode of action, it was assumed that carcinogenicity would be mediated by non-genotoxic (epigenetic) mechanisms.

There are no human data on reproductive toxicity. Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes (dose levels of 175, 300, and 700 mg/kg bw/d). The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment than that of females. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. With respect to developmental toxicity, it appears on the basis of the available data, that tris(2-chloroethyl) phosphate has no embryo-/fetotoxic or specific teratogenic properties even at maternally toxic doses. An oral NOAEL/developmental toxicity of 200 mg/kg bw/d (NOAEL/maternal toxicity = 100 mg/kg bw/d) was derived from studies with rats.

4.1.3.2 Workers

4.1.3.2.1 General aspects of occupational risk assessment

Tris(2-chloroethyl)phosphate (TCEP) is a liquid substance with a vapour pressure of 10^{-3} Pa at 20°C which is soluble in water and organic solvents. It is mainly used as plasticizer for polymers with flame-retarding properties. The occupational exposure scenarios have been described and discussed in section 4.1.1.2. Exposure routes to be considered at the workplace are inhalation against TCEP vapours, inhalative exposure to aerosols and skin contact with the liquid substance and its formulations.

The toxicological data of TCEP have been described and discussed in section 4.1.2. For each endpoint the specific experimental threshold level identified during hazard assessment will be taken forward for occupational risk assessment. Quantitative human toxicity data are not available, therefore risk considerations and estimations have to be based on animal data which have to be extrapolated accordingly. Default values concerning physiological parameters used during this procedure are listed below. From the toxicity profile of TCEP the most prominent effects seem to be carcinogenicity and repeated dose toxicity.

body weight, rat	250 g
body weight, human	70 kg
respiratory rate, rat at rest	0.8 l/min/kg
respiratory volume worker during 8h of light activity)	10 m^3

Systemic availability for different routes of exposure

For the majority of toxicological endpoints TCEP data originate from oral studies. Since workers are exposed either by inhalation or by skin contact, route to route transformation is essential for worker risk assessment. It is recognized though, that route to route extrapolation is associated with a high degree of uncertainty.

According to toxicokinetic data TCEP seems to be readily absorbed via the oral route. No data is available concerning absorption after inhalation or dermal application. For TCEP,

acute oral, dermal and inhalative studies are available, indicating a relatively low toxic potency following acute dermal application. Because comparison of results from acute toxicity studies generally gives very limited information concerning the systemic availability at relatively low doses (ECB, 2000), acute toxicity studies are not used to conclude on route-specific absorption for other toxicological endpoints. As a default assumption a complete absorption (100%) at all routes is assumed which is to some extent consistent with the physicochemical properties of the substance (MW < 500, solubility in water and organic solvents, $Log_{pow} = 1.7$).

Occupational exposure and internal body burden

In order to assess risks by combined exposure a socalled "internal body burden" is identified. This parameter combines the different contributions of dermal and inhalative exposures of workers to a total effective dose. For TCEP an approach weighed by absorption is not necessary because systemic availability is assumed to be similar at both routes (see above).

In table 4.1.3.2.A the route specific exposure values are listed and the internal body burdens of workers as result of repeated combined exposure via inhalation and dermal exposure are identified. The highest exposure level is obtained for scenario 3a, with a total internal body burden of up to about 2500 mg/person/day.

Short term exposure levels are not available. A calculation of short term exposure levels is not considered necessary, because there are no data which characterise TCEP as respiratory irritant. Inhalation toxicity of TCEP is assumed to depend rather on dose than on concentration.

Table 4.1.3.2.A: Occupational exposure levels and internal body burden of workers

Exposure scenario		Inhalation shift average (mg/m^3)	Dermal contact shift average (mg/p/d),	Internal body burden of workers after repeated exposure (mg/p/d)		
				Inhalation(1)	Dermal(2)	Combined
1	Production	1.2	420	12	420	432
2	Processing to formulations	1.2	420	12	420	432
3a	Use of formulations with spray application	8.3	2500	83	2500	2583
3b	Use of formulations without aerosol formation	1.2	210	12	210	222

(1) based on the assumption of 100% inhalative absorption; breathing volume of 10 m^3 per shift
(2) based on the assumption of 100% systemic availability of TCEP after dermal contact

Calculation of MOS values

For toxicological endpoints with quantitative data available, MOS values are calculated as quotient of experimental NOAEL (or LOAEL) from animal studies and workplace exposure assessments. For TCEP, oral doses from experimental studies are converted to air concentrations or dermal exposure levels before calculation of scenario-specific MOS values. For this procedure the physiological default values from above are used to modify the dose unit of effects data. As result a socalled “starting point” for risk assessment is identified.

MOS values for inhalative and dermal route are considered separately. The combined MOS value is calculated as quotient of the internal NAEL (or LAEL) and internal body burden of workers. Because 100 % absorption at all routes is assumed for TCEP (see above) the internal NAEL is supposed to be similar to the external NOAEL. In case of acute toxicity, different NOAELs for different application routes are available for TCEP.

With respect to the possible outcome of an assessment for combined risks, interest focusses on scenarios with conclusion ii at both exposure routes. By theoretical considerations combined exposure will not increase the most critical route-specific risk component more than twice. It is recognized on that background, that combined risks only rarely will decide concern. For matters of completeness however, all combined MOS values are given in this report on TCEP.

Evaluation of MOS values

Risk assessment based on MOS values implies the identification of a minimal MOS as decision mark between conclusion ii and iii. To obtain this, assessment factors are identified for TCEP, which vary depending on data availability and the specific toxicological endpoint to be evaluated. Scientifically based adjustment factors describe the extrapolation of animal data to the worker population. The uncertainties in the specific calculations are weighed by expert judgement and expressed as an additional “uncertainty factor”. The value of the minimal MOS results from the multiplicative combination of the different assessment factors. For carcinogenic risk assessment of TCEP, a modified MOS approach is used.

If the MOS value for a certain exposure scenario is below the minimal MOS for a specific endpoint, the corresponding risk situation is considered to be of concern. A MOS value higher than the minimal MOS indicates no concern.

In a parallel procedure, which gives identical but more direct results, the toxicological starting point taken forward to risk characterisation may be divided by the endpoint-specific assessment factors. As result an exposure level is identified for TCEP which by direct comparison with the occupational exposure levels may serve as trigger for decisions. In the context of this risk assessment report it will be called “critical exposure level”. Concern will be expressed for scenarios above this trigger value.

Interspecies differences

No information is available which allows to describe human susceptibility in comparison to that of experimental animals. As default approach worker risk assessment will therefore be based on the data from the most sensitive experimental species for a certain endpoint. Interspecies extrapolation is then performed using the concept of metabolic rate scaling.

For interspecies extrapolation of oral or dermal data metabolic rate scaling results in lower effective dose levels in mg per kg bodyweight for humans compared to experimental animals. For mice the scaling factor is 7, for rats 4 and for rabbits 2.2 (NO_NL, 1999). The scaling factor is calculated by the formula $(BW_{human}/BW_{animal})^{0.25}$.

For inhalation the principle of metabolic rate scaling implies that a specific inhalation exposure level (in mg/m^3) is toxicologically equivalent in experimental animals and humans. However, care has to be taken to rely the extrapolation between species on directly comparable conditions: under study conditions rats are thought to be at a state of reduced activity; the according human breathing volume in 8 hours is 6.7 m^3 (0.2 l/min/kg x 60min/h x 8h x 70 kg). Workers, however, are assumed to breathe 10 m^3 during a normal working day under conditions of light to moderate activity. Thus for workers the amount of substance inhaled must be spread over a 1.5 times higher breathing volume. Maintaining toxicological equivalence means that compared to the experimental levels the according occupational air concentrations will be 1.5 times lower.

Intraspecies differences

Humans differ in sensitivity due to biological factors. The actual risks for a single person may either be less or more pronounced than estimated for the average human. It is recognised that in order to cover the most sensitive person a very high default assessment factor would be required.

Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%). Using a lower factor of 3, for instance, would be protective for about 90% of the population whereas a factor of 10 would include 99% of the population. The empirical data do not allow to decide if a lower factor would be sufficient for certain toxicological effects, like for instance local effects in the airways. In the absence of further specific information the factor of 5 is used as default for all toxicological endpoints.

Duration adjustment

According to the fact that studies with suitable experimental design are available for TCEP there is no need for a specific duration adjustment step in extrapolation. Where adaptation of daily or weekly doses is necessary, adjustment is based on linearity.

Uncertainty considerations

The default adjustment factors outlined above are based upon evaluation of literature data for different chemicals. From a statistical point of view the individual parameters have to be understood as point estimates belonging to probability density distributions. Each factor is taken as geometric mean (point near the maximum) from its density function. The multiplicative combination of all factors is therefore supposed to result in a central tendency estimate. It addresses a likely situation for that percentile of the population reflected by the intraspecies factor.

To complete the assessment, the uncertainty included in the procedure outlined above should be addressed and, if necessary, used to modify the minimal MOS in terms of precaution. On that purpose several aspects should be taken into account, which by their nature are not easy to quantify. Examples are the reliability of the data base, the variability in assessment factors, the different steps necessary to bridge data gaps, the biological relevance of the observed effects.

4.1.3.2.2 Endpoint-specific risk assessment for worker

Acute Toxicity

Acute toxicity by inhalation

In an acute inhalation limit test rats were exposed to a nominal concentration of 25.7 mg/l for 1 hour, generated by means of a midget impinger. Rats showed moderate lacrymation and salivation without mortality. As starting point for MOS calculation the air concentration of 25700 mg/m^3 is chosen which did not lead to severe acute effects in rats.

Evaluation of the MOS values has to account for the following aspects: (a) study duration was 1 hour compared to occupational exposure of 8 hours, (b) physiological differences between humans at rest and workers account for a factor of 1.5, (c) the starting point for risk characterisation is some sort of an acute LOAEC which is transfered to an acute NOAEC with a factor of 3, (d) human intraspecies variation is accounted for by a factor of 5, (e) a further uncertainty factor of 2 is proposed because the LOAEL is based on a nominal (not measured) air concentration which gives indication that the risk situation might be more critical than estimated. Altogether the minimal MOS calculates to 360 (8/1 x 1.5 x 3 x 5 x 2). The critical exposure level is identified as 70 mg/m^3 (25700 mg/m^3 / 360). It is recognized that the quality of the acute inhalation study is rather limited. Nevertheless, this assessment of acute inhalation toxicity to some degree is consistent with the results of oral toxicity testing (see below in the chapter on acute toxicity by combined exposure).

The highest shift average value for inhalation is 8.3 mg/m^3 for spray application (scenario 3a). The according MOS value calculates to 3096 (25700 / 8.3), which, in comparison to the minimal MOS of 360, does not give reason for concern.

Acute toxicity by dermal contact

In a limit test in rabbits with occlusive exposure for 24 hours a dose of 2150 mg/kg did not result in apparent signs of toxicity. As starting point for MOS calculation the human dose corresponding to the dermal NOAEL in rabbits is calculated to 150500 mg/person (2150 mg/kg x 70 kg).

Evaluation of the MOS values has to account for the following aspects: (a) metabolic rate scaling from rabbits to humans reveals a factor of about 2, (b) human intraspecies variation is accounted for by a factor of 5, (c) against the background of rather stringent exposure conditions in the dermal toxicity study a further uncertainty factor is not considered necessary. Altogether the minimal MOS calculates to 10 (2 x 5). Based on the result of acute dermal toxicity testing the critical exposure level is identified as about 15000 mg/person (150500 mg/person / 10).

The highest dermal exposure level is reported to be up to 2500 mg/person for spray application (scenario 3a). The according MOS value calculates to 60 (150500 / 2500) which, in comparison to the minimal MOS of about 10, does not give reason for concern (compare table 4.1.3.2.B).

Acute toxicity by combined exposure

There may be different starting points for characterisation of acute toxicity by combined exposure. As well the quality of the diverse acute toxicity data for the different application ways as the relationship in potency of the different routes of exposure should be taken into account: Acute dermal toxicity is clearly lower (dermal limit test in rabbits, exposure for 24 hours of about 2000 mg/kg without apparent sings of toxicity, see above) than acute oral toxicity (LD50 in rats is about 1000 mg/kg, 800 mg/kg are without lethality) and inhalative toxicity (moderate lacrymation and salivation without lethality at 1,200 mg/kg in rats (calculation: with a breathing volume of 0.8 l/min/kg a rat breathes 48 l in 1 hour. If 25 mg/l TCEP are in the air, the rat breathes 1,200 mg/kg (25mg/l x 48 l/hour)).

Risk assessment for acute toxicity by combined exposure starts with oral toxicity studies keeping in mind, that the resulting calculation might be probably conservative. In a rat study a LD50 between 1000 and 1260 mg/kg was calculated. At 800 mg/kg clinical signs as pilo-erection and salivation are reported, but no mortality. It might be additionally helpful to have a look at the results of the developmental oral toxicity study by Kawashima et al. (1983). Pregnant dams were orally exposed (by gavage) from day 7 to 15 of pregnancy. While 200 mg/kg/d led to mortality, no changes in maternal body weight gain, food consumption and general appearance resulted from oral application up to 100 mg/kg/d. For oral application, 100% absorption is assumed.

Based on this oral (internal) starting point of 100 mg/kg/d (7000 mg/p/d) the evaluation of scenario-specific MOS values has to account for the following aspects: (a) metabolic rate scaling is reflected by a factor of 4, human intraspecies variation is described by a factor of 5, the acute NOAEL is considered to be higher than the subacute NOAEL used as starting point (factor 1/3). Altogether, the minimal MOS calculates to about 7 (4 x 5 x 1/3).

There is only one occupational scenario (spray application, scenario 3a) for which the scenario-specific MOS (of 3) is lower than the minimal MOS (of 7). Comparison of the results of acute toxicity for the various routes of application shows that acute oral toxicity is much more pronounced than acute dermal toxicity (see above). Recognizing that the internal exposure in scenario 3a is nearly totally governed by dermal contact, conclusion ii for

scenario 3a for combined exposure is warranted. This decision is consistent with the decision for scenario 3a for dermal contact alone which is based on an acute dermal toxicity study rather than on acute oral testing (table 4.1.3.2.B).

Conclusion: ii

Table 4.1.3.2.B: MOS values for acute toxicity of TCEP, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	25700 mg/m³			150500 mg/p/d			7000 mg/p/d		
Minimal MOS	360			10			7		
Critical exposure level	70 mg/m³			15000 mg/p/d			1000 mg/p/d		
	Exposure (mg/m³)	MOS	Conclusions	Exposure (mg/p/d)	MOS	Conclusions	Internal body burden (mg/p/d)	MOS	Conclusions
1 Production	1.2	21417	ii	420	358	ii	432	16	ii
2 Processing to formulations	1.2	21417	ii	420	358	ii	432	16	ii
3a Use of formulations with spray application	8.3	3096	ii	2500	60	ii	2583	3	ii(1)
3b Use of formulations without aerosol formation	1.2	21417	ii	210	717	ii	222	32	ii

(1) conclusion ii was reached although MOS is lower than minMOS; explanation see text

Irritation/Corrosivity

Dermal and eye irritation

In studies with rabbits slight dermal erythema and weak irritation of the conjunctivae was observed for TCEP. The effects are not sufficient for classification. There is no concern from dermal or eye irritation at the workplace.

Conclusion: ii

Acute respiratory irritation

No studies are available concerning the irritation potential of TCEP after inhalation. From irritation studies at skin or eyes comes no indication that the substance may cause serious effects at the site of initial contact. A risk relevant damage of the airways by acute irritation properties is therefore not anticipated. There is no reason for concern.

Conclusion: ii

Sensitisation

Skin sensitisation

Human data on sensitizing properties of the substance are not available.

The skin sensitisation potential of TCEP was evaluated with the Buehler method at Guinea pigs (Mobil (1983). No allergic reactions were seen. Additionally the test results of two other structurally related chloroalkyl phosphate esters (guinea pig maximisation tests) gave no evidence to skin sensitising properties. Based on all information on the structurally related chloroalkyl phosphates (results of animal testing, similarity in physicochemical data and chemical structures, as well as alkylating properties of TCEP, TCPP and TDCP) it is concluded that TCEP should be non-sensitizing to humans. There is no reason for concern.

Conclusion: ii

Respiratory sensitisation

No information is available on the respiratory sensitisation potential of TCEP. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. Considering that the production of TCEP is performed in closed systems and that TCEP has a low vapour pressure one would expect that exposure of the respiratory tract is low. However, TCEP is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all the years of use no notice of specific case reports has been given. There is no concern from respiratory sensitisation at the workplace.

Conclusion: ii

Repeated dose toxicity

Local effects (RDT) by inhalation or dermal contact

In a rabbit study TCEP caused slight irritation (dermal, eye). There is no information on irritation potency following acute or repeated exposure and there are no experimental studies or reported experiences concerning respiratory tract irritation following acute or repeated exposure. Against that background, conclusion ii is reached. However, it should be mentioned, that on the available basis of acute irritation data no valid predictions can be made about the irritation potency following repeated exposure (Rennen et al. 2002).

Conclusion: ii

Systemic effects (RDT) by inhalation, dermal contact and combined exposure

Several studies with repeated application, mainly by the oral route, have been performed in mice and rats. Primary target organs turned out to be the kidneys, the brain and the liver. The effects are expressed to a different degree in mice and rats, additionally male and female animal appear to be differently susceptible.

The LOAEL of 12 mg/kg bw/d for kidney lesions in Scl:ddY mice is chosen as the basis for risk assessment. Mice fed ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months developed hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei cysts (Takada et al. 1989). This LOAEL is the lowest LOAEL from different oral chronic toxicity studies with TCEP. The according human dose calculates to 840 mg/person/day (12 mg/kg/d x 70 kg), the corresponding occupational air concentration for 8-hour inhalation is identified as 84 mg/m^3 (840 mg/person/day / 10 m^3/day). These values are used as starting points for MOS calculations.

Evaluation of the MOS values has to account for the following aspects: (a) a LOAEL-to-NAEL extrapolation factor of 3 is proposed, (b) assessment starts from a mouse dose scaled per body weight, thus for species extrapolation from mice to humans a factor of 7 is introduced, (c) the human intraspecies variation is accounted for by a factor of 5, (d) experimental dosing was 7 days per week compared to occupational exposure of 5 days per week, (e) no furhter uncertainty factor is proposed. Altogether the minimal MOS for systemic effects after repeated exposure calculates to 75 (3 x 7 x 5 x 5/7). The according critical exposure level is 10 mg/person/day (840 mg/person/day / 75) which is used for the assessment of dermal contact (external dose) and combined exposure (internal dose). The corresponding critical exposure level for inhalation is 1 mg/m^3 (84 mg/m^3 / 75).

As can be derived from the data in table 4.1.3.2.C conclusion iii is reached for all occupational exposure scenarios for all routes of exposure. However, those occupational scenarios with inhalation exposure levels of 1.2 mg/m³ are considered to be borderline situations.

The most critical risk situation seems to be dermal exposure during spray application (scenario 3a). For dermal scenarios 1, 2, 3a and 3b, the scenario-specific MOS values for RDT are less than 1/10 of the minimal MOS. This clear difference should be taken into account, when considering the consequence of a dermal absorption of possibly somewhat less

than 100% on conclusions for dermal risk assessment. Handling sticky resins (scenario 3c) leads to concern as well, but is considered less critical compared to the other dermal scenarios.

Conclusion: iii

Table 4.1.3.2.C: MOS values for repeated dose toxicity of TCEP, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	84 mg/m³			840 mg/p/d			840 mg/p/d		
Minimal MOS	75			75			75		
Critical exposure level	1 mg/m³			10 mg/p/d			10 mg/p/d		
	Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1 Production	1.2	70	iii	420	2	iii	432	2	iii[1)]
2 Processing to formulations	1.2	70	iii	420	2	iii	432	2	iii[1)]
3a Use of formulations with spray application	8.3	10	iii	2500	0.3	iii	2583	0.3	iii[1)]
3b Use of formulations without aerosol formation	1.2	70	iii	210	4	iii	222	4	iii[1)]

[1)] conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Mutagenicity

In vitro and in vivo tests have been performed to investigate the genotoxic properties of TCEP. In summary there is no relevant evidence for mutagenicity of TCEP. There is no reason for concern.

Conclusion: ii

Carcinogenicity

Carcinogenicity by inhalative, dermal and combined exposure

Carcinogenicity studies by gavage conducted to modern regulatory standards are available in rats and mice. These studies provide clear evidence that TCEP is carcinogenic in experimental animals. In the rat, TCEP caused an increased incidence of renal tubule adenomas in males and females at 44 mg/kg/day and above. In addition, thyroid follicular cell neoplasms and mononuclear cell leukemia may have been related to TCEP administration. In a comparable study with B6C3F1 mice, evidence for carcinogenicity was shown by a slightly increased incidence of tubule cell neoplasms obtained in supplemental evaluation of additional kidney sections. In a further experiment with Scl:ddY mice a significant increase of kidney and liver tumours occurred at 300 mg/kg/day and above. The detailed tumour incidences are summarized in chapter 4.1.2.8.

From dermal short-term tests there is no indication for a carcinogenic, promoting or initiating potential of TCEP on mouse skin. However limitations in study design and reporting do not allow firm conclusions to be drawn from the dermal studies. There is no inhalative study. Thus for the both exposure routes which are relevant at the workplace no route-specific reliable information is available concerning the endpoint carcinogenicity. As default approach the oral data are used for risk assessment at the workplace, which clearly demonstrate the potential of TCEP to induce neoplasm.

There is no evidence of a direct genotoxic mode of action. For carcinogenic risk assessment it is assumed that carcinogenicity is mediated by a non-genotoxic (epigenetic) mechanism. At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in secondary formation of tumours (see discussion in chapter 4.1.2.8). Recognizing the scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP, for occupational risk assessment it is nevertheless considered adequate and justifiable to give risk managers some additional practical guidance on the carcinogenic potency of TCEP. It seems possible, that one of the adverse effects described in the TCEP reports on chronic toxicity might be responsible for the induction of tumour development. Thus, risk assessment for repeated dose toxicity might give some additional guidance. If any of the reported chronic effects of TCEP is responsible as starting point for TCEP tumour development, an increase of tumour incidence is not anticipated to occur up to the critical exposure levels for repeated dose toxicity (see preceding chapter). This approach implies that the LOAEL for kidney lesions in mice is used as starting point for carcinogenic risk assessment as well.

The MOS approach for repeated dose toxicity is proposed to be modified for carcinogenic risk assessment. The main reason is, that the degree of adversity of neoplasms is considered to be much more pronounced than the degree of adversity of non-neoplastic adverse effects. This consideration supports the introduction of an additional extrapolation factor for carcinogenity in order to increase the level of protection.

On a practical basis, it is proposed to introduce an additional adjustment factor of 5. This approach results in a minimal MOS for carcinogenicity of 375 (75 x 5) and the critical exposure levels of 0.2 mg/m³ (inhalation) and 2 mg/p/d (dermal contact).

A sound justification of the amount of this additional adjustment factor of 5 is difficult. However, it might be possible to evaluate the consequences of the proposed approach by the following theoretical considerations. According to Dybing et al. (1977) a T25 value for TCEP is calculated. Based on the overall rates for kidney adenomas and carcinomas in the rat carcinogenicity study a T25 of 130 mg/kg/day is obtained (44 mg/kg/d x 25% / 6% x 5d/7d x 103wk/104wk). In case of a linear dose-response relationship (which is not valid for TCEP) the corresponding level of risk for the proposed critical exposure level of 2 mg/p/d (or 0.029 mg/kg/d) can be easily calculated (0.25 x 0.029/130). According to this calculation, the proposed critical exposure level of 0.2 mg/m³ (inhalation) and 2 mg/p/d (dermal contact) would be connected with a risk level of about 6:100.000 in case of (non-existing) linearity. Accounting for species extrapolation and differences in exposure schedule does not substantially modify this dimension of risk. Against that background of pragmatic considerations, being aware of the scientific uncertainties involved, carcinogenic risk assessment for TCEP is proposed to be based on that modified MOS approach for repeated dose toxicity (see table 4.1.3.2.D).

As can be derived from the data in table 4.1.3.2.D for carcinogenicity conclusion iii is reached for all occupational exposure scenarios for all routes of exposure. The MOS approach used for carcinogenic risk assessment for TCEP indicates that occupational exposure levels in all three scenarios have to be reduced substantially. The most critical risk situation seems to be dermal exposure during spray application (scenario 3a). Assuming limited protection from wearing suitable gloves (e.g. 90% protection), it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Conclusion: iii

Table 4.1.3.2.D: Estimation of MOS values for cancer risks by TCEP

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		84 mg/m³			840 mg/p/d			840 mg/p/d		
Minimal MOS		375			375			375		
Critical exposure level		0.2 mg/m³			2 mg/p/d			2 mg/p/d		
		Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1	Production	1.2	70	iii	420	2	iii	432	2	iii[1)]
2	Processing to formulations	1.2	70	iii	420	2	iii	432	2	iii[1)]
3a	Use of formulations with spray application	8.3	10	iii	2500	0.3	iii	2583	0.3	iii[1)]
3b	Use of formulations without aerosol formation	1.2	70	iii	210	4	iii	222	4	iii[1)]

(1) conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Reproductive toxicity, fertility impairment

Fertility impairment by inhalation, dermal and combined exposure

In an oral study with a continuous breeding protocol TCEP treatment revealed impairment of fertility in mice. At 700 mg/kg/day significant reduction of the number of litters produced by the F0 generation was observed. The findings were confirmed with a separate cross over mating trial at the same dose level. The reproductive system of male mice seemed to be more sensitive to the TCEP treatment than that of female mice. A NOAEL of 175 mg/kg/day is derived from the study (see chapter 4.1.2.9). As starting point for MOS calculation the human dose according to the experimental NOAEL is identified as 12250 mg/person/day (175 mg/kg/d x 70 kg), the corresponding occupational air concentration for 8 hours inhalation calculates to 1225 mg/m³.

In evaluation of the MOS values the following aspects have to be considered: (a) metabolic rate scaling from mice to humans yields a factor of 7, (b) for human intraspecies variation a factor of 5 is used, (c) dosing was 7 days per week in the study compared to occupational exposure of 5 days per week, which should be accounted for, (d) an uncertainty factor of 3 is proposed because the nature of the effects is judged to be severe and there is indication for a

steep dose response relationship. Together the minimal MOS calculates to 75 (7 x 5 x 5/7 x 3). The according critical exposure level is 160 mg/person/day (12250 mg/person/day / 75) or 16 mg/m^3 (1225 mg/m^3 / 75). It has to be noticed that these values lie above the critical exposure level for repeated dose toxicity.

In table 4.1.3.2.E MOS values and conclusions for fertility impairment of TCEP are listed in detail. Concern is expressed for dermal exposure scenarios (scenarios 1, 2, 3a and 3b). Handling sticky resins (scenario 3c) results in conclusion ii.

With respect to fertility impairment risk reduction measures at the workplace appear to be necessary. However, it should be kept in mind, that as a consequence of risk assessment with respect to repeated dose toxicity and carcinogenicity, risk reduction measures will have to be requested, which efficiently reduce occupational exposures to a level preventing workers from the according effects. If these control measures are implemented and complied with, exposures will be brought to a level which is below concern with respect to fertility impairment too.

Conclusion: iii

Table 4.1.3.2.E: MOS values for fertility impairment of TCEP

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		1225 mg/m³			12250 mg/p/d			12250 mg/p/d		
Minimal MOS		75			75			75		
Critical exposure level		16 mg/m³			160 mg/p/d			160 mg/p/d		
		Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1	Production	1.2	1021	ii	420	29	iii	432	28	iii[1)]
2	Processing to formulations	1.2	1021	ii	420	29	iii	432	28	iii[1)]
3a	Use of formulations with spray application	8.3	148	ii	2500	5	iii	2583	5	iii[1)]
3b	Use of formulations without aerosol formation	1.2	1021	ii	210	58	iii	222	55	iii[1)]

[1)] conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Reproductive toxicity, developmental effects

Developmental effects by inhalative, dermal and combined exposure

No significant toxicity to embryo or fetal development has been observed after oral TCEP treatment of pregnant rats. Concerning developmental toxicity the NOAEL from this study is reported as 200 mg/kg/day, a dose which clearly induced maternal toxicity. Based on the available data, the results are interpreted as giving no indication for developmental toxicity of TCEP up to doses which induce chronic toxic effects and/or carcinogenicity. There is no concern with respect to this endpoint for workers.

Conclusion: ii

4.1.3.2.3 Summary of occupational risk assessment

Table 4.1.3.2.F indicates the toxicological endpoints of concern for TCEP. There is concern for fertility impairment, repeated dose toxicity and for carcinogenicity.

Table 4.1.3.2.F: Endpoint-specific overall conclusions

Toxicological endpoints		general conclusion
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	ii
	respiratory	ii
Repeated dose toxicity	inhalation, local	ii
	inhalation, systemic	iii
	dermal, local	ii
	dermal, systemic	iii
	combined, systemic	iii[1)]
Mutagenicity		ii
Carcinogenicity	inhalation	iii
	dermal	iii
	combined	iii[1)]
Fertility impairment	inhalation	ii
	dermal	iii
	combined	iii[1)]
Developmental toxicity	inhalation	ii
	dermal	ii
	combined	ii

[1)] conclusion iii already results from from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

In table 4.1.3.2.G occupational exposure scenarios are listed in the order of scenario numbers to give an overview for all exposure situations with concern. All toxicological endpoints are listed, which at least in one case give reason for conclusion iii. Under this aspect acute toxicity, irritation, dermal and respiratory sensitisation, and developmental toxicity are not included in table 4.1.3.2.G. For TCEP concern in general results from inhalation and from dermal contact to the substance.

Table 4.1.3.2.G: Summary of exposure scenarios with concern for TCEP [1]

Exposure scenarios		Repeated dose toxicity			Carcino-genicity			Fertility		
		inhalation	dermal	combined [2]	inhalation	dermal	combined [2]	inhalation	dermal	combined [2]
1	Production	iii	iii	iii	iii	iii	iii		iii	iii
2	Processing to formulations	iii	iii	iii	iii	iii	iii		iii	iii
3a	Use of formulations with spray application	iii	iii	iii	iii	iii	iii		iii	iii
3b	Use of formulations without aerosol formation	iii	iii	iii	iii	iii	iii		iii	iii

[1] blanc fields: conclusion ii

[2] conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Risk estimation is mainly based on oral studies. Because no data are available concerning absorption after inhalation or skin contact, route to route extrapolation is based on the default assumption of 100 % absorption at all routes.

The most important toxicological endpoint is TCEP carcinogenicity in combination with repeated dose toxicity. On the background of the exposure assessment and the proposed critical exposure levels, the according health risks are comparably high. This evaluation of risk is based on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

Tables 4.1.3.2.H (inhalation) and 4.1.3.2.I (dermal contact) try to visualize the risk profile of TCEP. According to the specific arrangement of exposure scenarios and critical exposure levels for different toxicological endpoints you will find the relatively high risks in the left upper corner, the relatively low risks in the bottom right corner of the tables.

On the background of cancer risks, air concentrations of TCEP at the workplace should be controlled to a level in the range of 0.2 mg/m^3 (critical exposure level for carcinogenicity). By this exposure reduction inhalative risks from other endpoints, as repeated dose toxicity and fertility impairment would similarly and effectively be mitigated too. Special emphasis should be given to reduce skin contact with TCEP (even if dermal absorption proved to be somewhat lower than 100%). Based on cancer risk assessment, dermal exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce dermal exposure.

Table 4.1.3.2.H: Ranking of the critical exposure levels for TCEP with respect to inhalative exposure at the workplace

Exposure scenario		Exposure level in mg/m³	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/m³			
			0.2 mg/m³	1 mg/m³	16 mg/m³	70 mg/m³
3a	Use of formulations with spray application	8.3	iii	iii		
1	Production	1.2	iii	iii		
2	Processing to formulations	1.2	iii	iii		
3b	Use of formulations without aerosol formation	1.2	iii	iii		

(1) blank fields: conclusion ii

Table 4.1.3.2.I: Ranking of the critical exposure levels for TCEP with respect to dermal exposure at the workplace

Exposure scenario		Exposure level in mg/p/d	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/p/d			
			2 mg/p/d	10 mg/p/d	160 mg/p/d	15000 mg/p/d
3a	Use of formulations with spray application	2500	iii	iii	iii	
1	Production	420	iii	iii	iii	
2	Processing to formulations	420	iii	iii	iii	
3b	Use of formulations without aerosol formation	210	iii	iii	iii	

(1) blank fields: conclusion ii

4.1.3.3 Consumers

Exposure

Tris(2-chloroethyl) phosphate (TCEP) is used as a flame retardant in materials that may come in contact with consumers. Several reports have shown that TCEP will be released from a number of sources which have been treated with flame retardants, namely timber, foam rubber, carpets, plastic materials (e.g. electronic devices, TV, car interior etc.), glues, and lacquers. Upholstery may sometimes be proved with flame retardants. It is released primarily by abrasion and becomes part of the dust fraction. Dust burden therefore reflects the sum of all the sources house dust and airborne dust.

Oral exposure can be referred to dust intake, due to hand-to-mouth behaviour, contamination of articles for daily use, e.g. toys. This pathway of exposure may play a particular role for children. Inhalation exposure takes place by inhaling airborne particles, and dermal exposure can occur from direct contact with e.g. furniture coverings as well as with house dust and airborne dust.

Inhalation

The measurements performed by Ingerowski et al. (2001) resulted in a 98th percentile for air concentrations of TCEP of 0.6 µg/m³**.** This value lies at the extreme upper range of a huge number of measurements. Although other studies have revealed lower values, this value was taken as a reasonable worst case for calculating the body burden.

The model estimate revealed an uptake by inhalation (99th percentiles) of TCEP of 0.4 and 0.96 µg/kg bw/d for adults and for children, respectively, under consideration of 100% absorption.

Dermal

Dermal exposure due to migration of tris(2-chloroethyl) phosphate from upholstery accounts for 3.9 µg/kg bw/d under worst case conditions (100% dermal absorption). Dermal exposure from airborne dust can be neglected due to the low concentrations of TCEP in dust (max. 0.1 µg/m³). Dermal exposure from house dust was calculated to amount to 0.02 µg/kg bw/d for children.

Dermal exposure from different sources can be estimated to a total of about 4 µg/kg bw/d for adults. Dermal exposure of children as related to bodyweight (10 µg/kg bw/d) can exceed that in adults.

Oral

Oral exposure of TCEP is characterised by dust uptake. The estimated amount accounts 0.0033 µg/kg bw/d for an adult, representing the 99th percentile. This pathway may represent a significant source of exposure for children, the 99th percentile representing daily uptake for a three year old child was estimated to be 0.2 µg/kg bw/d.

For babies of about 3 months a significant source could be sucking at a toy. Under worst case assumptions exposure values can be estimated up to 240 µg/kg bw/d.

Total body burdens

For female adults, a body burden would account for ~ 4.5 µg/kg bw/d, under reasonable worst case conditions and taking all paths into consideration. The respective value for 1 - 3 year-old children is 11 µg/kg bw/d. For babies of about 3 months a body burden would account up to 240 µg/kg bw/d by sucking on toys, the other paths can be neglected.

Effects

Acute Toxicity

Following exposure assessment, consumers are expected to be exposed to tris(2-chloroethyl) phosphate several orders of magnitude lower than the range of doses which can be derived from acute oral toxicity based on animal LD50 values (oral, rats: 430-1230 mg/kg bw). In an experiment with rabbits, the substance has demonstrated low dermal toxicity, in experiments with rats, low acute inhalation toxicity. Therefore, the substance is of no concern for the consumer in relation to acute oral, inhalation or dermal toxicity.

Conclusion: ii

Irritation

Data on human experience with tris(2-chloroethyl) phosphate are not available.

In experiments with rabbits, the substance demonstrated weak skin irritation after occlusive or semi-occlusive patch testing for 4 to 24 hours exposure times. After instillation into the eyes of rabbits, mild conjunctival irritation was detected; this irritation healed within 2 days.

Conclusion: ii

Corrosivity

Information on local effects on skin and eyes of humans is not available. Tris(2-chloroethyl) phosphate is not a corrosive substance, as judged on the basis of Draize tests with rabbits.

Conclusion: ii

Sensitisation

Human data on sensitizing properties of tris(2-chloroethyl) phosphate are not available. An animal study (Buehler Test) showed no skin sensitizing potential of TCEP. The read across from sensitisation data of the two other chloroalkyl phosphates TCPP and TDCP indicated that these substances do not possess significant skin sensitisation potential. Based on data on TCEP and structurally related substances it is concluded that there is no concern.

Conclusion: ii

Repeated dose toxicity

NOAELs for systemic effects on the brain were derived in each case from subchronic oral toxicity studies for rats at dose levels between 44 to 75 mg/kg bw/d. A NOAEL for kidney lesions was estimated only in subchronic toxicity studies for female Sprague-Dawley rats at 215 mg/kg bw/d and for B6C3F1 mice at 350 mg/kg bw/d. No NOAEL for kidney effects could be derived for male Sprague-Dawley rats fed ≥26 mg/kg bw/d in the diet for three months, and for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d. For mice the LOAELs for kidney lesions range between 12 to 175 mg/kg bw/d (for Scl:ddY mice after feeding in the diet for 18 months and for B6C3F1 mice after long-life exposure, respectively). Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl) phosphate the LOAEL for kidney lesions in Scl:ddY mice (12 mg/kg bw/d) was chosen as the basis for risk assessment.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

  There are no reasons to assume an important uncertainty.

- Overall confidence in the database

  There are no contradiction results. All listed studies were of sufficient data quality.

- Intra- and interspecies variation

  Results of studies give clear evidence that mice were more sensitive to the kidney effects than rats. Thus, the interspecies variation seems to be considerable. There is a single report in humans after high inhalation exposure of a child aged five years. An extremely rare genetic susceptibility may be discussed as the underlying cause for the neuromuscular disease which became apparent and disappeared after cessation of exposure.

- Dose response relationship

  There is no reason to assume a special concern.

- Nature and severity of the effect

  The adverse effects seen at the lowest observed adverse effect level are in kidneys and these effects are considered to be severe (irreversible).

  There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

  The estimated body burdens (with an assumed absorption of 100%) are compared with an oral LOAEL from an 18 month study.

  There are no reasons to assume that special concern can be derived neither from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies.

  Following the exposure considerations there is reason to assume a special risk for babies sucking a TCEP containing textile cube and for children due to TCEP exposure from house dust.

- Other factors

  There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenarios via different routes

Table 4.1.3.3
Repeated dose toxicity of TCEP:
Margins of Safety for consumer exposure via different routes

Route	Exposure (µg/kg bw/d)	MOS *)	Conclusion
Inhalation			
Adults	0.4	30000	(ii)
Children	0.96	12500	(ii)
Dermal			
Adults	~ 4	3000	(ii)
Children	10	1200	(ii)
Oral			
Adults	0.0033	$>3 \cdot 10^6$	(ii)
Children	0.2	60000	(ii)
Babies	240	50	(iii)

*) The MOS was derived by using the oral LOAEL of 12 mg/kg bw/d (kidney effects)

Repeated dose toxicity - Adults

For all exposure scenarios via different routes the margins of safety are judged to be sufficient taking into account that the absorption figures are reasonable worse case estimates and even that a LOAEL has been used.
Conclusion: ii

Repeated dose toxicity - Children

The margins of safety for children specific exposure via different routes are judged to be sufficient taking into account that the absorption figures are reasonable worse case estimates and even that a LOAEL has been used.
Conclusion: ii

Repeated dose toxicity - Babies

The margin of safety for babies due to sucking on toys is judged to be not sufficient to cover all uncertainties. It has to be taken into account that a NOAEL for kidney effects is not available. The use of a LOAEL for risk characterisation requires a higher margin of safety. Even in case of an underlying worst case exposure estimate the MOS of 50 is considered to be not sufficient thus leading to concern for repeated dose toxicity.

Conclusion: iii

Mutagenicity

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations. Two in vivo mammalian micronucleus tests and a Drosophila test were negative. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. There is the overall conclusion of no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate.

Conclusion: ii

Carcinogenicity

Tris(2-chloroethyl)phosphate induces the formation of benign and malignant tumors at various sites in experimental animals. Renal tubule cell neoplasms were found in rats and mice each in both sexes. At present the mechanism of tumor development in the kidneys of tris(2-chloroethyl) phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that TCEP appears to have no genotoxic potential. Thus it is likely that the tumor formation in the kidney would be mediated by non-genotoxic (epigenetic) mechanisms. A species specific mechanism of tumor formation in the kidney was not identified.

At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in formation of kidney tumours (see discussion in chapter 4.1.2.8). It seems possible, that any of the adverse effects described in the TCEP studies on chronic toxicity might be responsible for the induction of tumour development.

Although it is considered likely that tumour formation especially in the kidney would be mediated by a non-genotoxic (epigenetic) mechanism, a threshold level for carcinogenicity has not been established due to the lack of relevant data. Renal tumors in male and female F344/N rats, in male B6C3F1 mice and in male Scl:ddY mice may be induced secondary via cytotoxicity followed by cell proliferation. However, a NOAEL for the cytotoxic effects and also for a cell proliferation mechanism could not be derived.

The scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP have to be recognized. However, one could imagine that any of the adverse effects described in the studies on chronic toxicity of TCEP might be responsible for the induction of tumour development. Given this, the LOAEL for kidney lesions obtained in Scl:ddY mice after feeding of 12 mg/kg bw/d in the diet for 18 months might be used as a surrogate LOAEL for

risk assessment purposes on carcinogenicity using a MOS approach. Thus, it is therefore considered justified to perform the risk assessment of consumers on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

The total body burden for female adults estimated for reasonable worst case conditions and taking into account all paths of exposure (inhalation, dermal, and oral) would account for ~ 4.5 µg/kg bw/d. The respective value for 1 - 3 year-old children is calculated to be 11 µg/kg bw/d. Taking into account the underlying worst case exposure scenarios with assumed bioavailabilities of 100% for all exposure routes the margin of safety between the assumed oral LOAEL of 12 mg/kg bw/d (kidney lesions) and the estimated exposure levels is judged to be sufficient to conclude on no concern for tumor formation in consumers (MOS 2660) and children (MOS 1090), too.

Conclusion: ii

An oral body burden for babies (3 months) for worst case conditions was calculated to be 240 µg/kg bw/d. It has to be taken into account that a NOAEL for for tumor formation is not available. The use of a LOAEL for risk characterisation requires a higher margin of safety. Even taking into account the underlying worst case exposure scenario with an assumed bioavailability of 100% the margin of safety between the assumed LOAEL of 12 mg/kg bw/d and the exposure level is judged to be not sufficient with respect to a possibility of tumor formation (MOS 50).

Conclusion: iii

Toxicity for reproduction

- Fertility

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. Reproductive failure was observed at daily doses of 700 mg/kg bw with at best and no more than 3 litters produced and with no pups surviving from the last litter produced. At 700 mg/kg bw/d a significant decrease was noted with respect to right epididymal and right testicular weights. Furthermore, at this dose level the average sperm count per gramme caudal tissue was statistically significantly decreased and the incidence of abnormal sperm was statistically significantly elevated relative to the control values.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty.

- Overall confidence in the database

There are no reasons to assume no confidence.

- Intra- and interspecies variation

The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice.

- Differences in exposure (route, duration, frequency and pattern)

The estimated total body burden with an assumed oral and dermal absorption and absorption by inhalation of 100% is compared with an oral NOAEL.

There are no reasons to assume that special concern can be derived from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenarios there might be a special risk for babies and children due to group-specific exposure behavior.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenarios for different routes

The total body burden for female adults estimated for reasonable worst case conditions and taking into account all paths of exposure (inhalation, dermal, and oral) would account for ~ 4.5 µg/kg bw/d. The respective value for 1 - 3 year-old children is calculated to be 11 µg/kg bw/d. The margin of safety between the oral NOAEL (fertility) of 175 mg/kg bw/d and the estimated exposure levels in the µg range is judged to be sufficient to conclude on no concern for fertility to consumers (MOS 39000) and children (MOS 16000), too.

Conclusion: ii

A body burden for babies (3 months) for worst case conditions was calculated to be 240 µg/kg bw/d orally. The margin of safety between the oral NOAEL of 175 mg/kg bw/d and the estimated exposure level is judged to be sufficient to conclude on no concern for fertility to babies (MOS 730).

Conclusion: ii

- Developmental toxicity

Teratogenic effects of tris(2-chloroethyl) phosphate were investigated up to doses of 200 mg/kg bw/d. No embryo-/fetotoxic or specific teratogenic properties were observed even at maternally toxic doses (NOAEL/maternal toxicity = 100 mg/kgbw/d). Therefore, developmental toxicity is not considered to be a relevant endpoint.

Conclusion: ii

4.1.3.4 Man exposed via the environment

Exposure

Indirect exposure via the environment is calculated using data for intake via food, air and drinking water. Following the local scenario data (cf. 4.1.1.4) a total daily intake of TCEP for humans of 5.8 µg/kg bw/d is calculated. Calculations for the regional scenario resulted in a total daily dose of 1.1 x 10^{-5} mg/kg bw/d. The main route of exposure is the stem for both approaches.

Effects

Acute Toxicity

Following exposure assessment, exposure of humans via the environment is expected to be several orders of magnitude lower than the range of doses which can be derived from acute oral toxicity based on animal LD50 values (oral, rats: 430-1230 mg/kg bw). Therefore, the substance is of no concern for the consumer in relation to acute oral toxicity.

Conclusion: ii

Irritation

Data on human experience with tris(2-chloroethyl) phosphate are not available.

In experiments with rabbits, the substance demonstrated weak skin irritation after occlusive or semi-occlusive patch testing. After instillation into the eyes of rabbits, mild conjunctival irritation was detected. Given the oral route as predominant way of uptake there is no concern.

Conclusion: ii

Corrosivity

Information on local effects on skin and eyes of humans is not available. Tris(2-chloroethyl) phosphate is not a corrosive substance. Given the oral route as predominant way of uptake there is no concern.

Conclusion: ii

Sensitisation

There are no human data on sensitisation available. Based on available data on TCEP and structurally related substances which have been tested in animal tests and the fact that the oral uptake is the main route it is concluded that there is no concern.

Conclusion: ii

Repeated dose toxicity

NOAELs for systemic effects on the brain were derived subchronic oral toxicity studies for rats at dose levels between 44 to 75 mg/kg bw/d. A NOAEL for kidney lesions was estimated only for female SD rats at 215 mg/kg bw/d and B6C3F1 mice at 350 mg/kg bw/d. No NOAEL could be derived for male SD and for male and female F344/N rats (cf. 4.1.1.3). Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl) phosphate the lowest LOAEL for kidney lesions in Scl:ddY mice (12 mg/kg bw/d) was chosen as the basis for risk assessment.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

  There are no reasons to assume an important uncertainty.

- Overall confidence in the database

  There are no contradiction results. All listed studies were of sufficient data quality.

- Intra- and interspecies variation

  Results of studies give clear evidence that mice were more sensitive to the kidney effects than rats. Thus, the interspecies variation seems to be considerable. There is a single report in humans after high inhalation exposure of a child aged five years.

- Dose response relationship

  There is no reason to assume a special concern.

- Nature and severity of the effect

  The effects described as low observed adverse effect are effects in kidneys, these effects are considered to be severe (irreversible).

  There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

  The calculated total daily doses are compared with an oral LOAEL from an 18 month study.

There are no reasons to assume that special concern can be derived neither from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies.

There is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenario:

Man exposed indirectly via the environment

Local scenario

The calculated total daily intake 0.0058 mg/kg bw/d is compared with an oral LOAEL of 12 mg/kg bw/d. The margin of safety of 2069 is judged to be sufficient taking into account considerations on intra- and interspecies variation, nature and severity of the effects and that a LOAEL is used for derivation of the MOS. Thus, the substance is considered to be of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The regional total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety of about 10^6 is judged to be sufficient. Thus, there is no concern in relation to regional exposure via the environment.

Conclusion: ii

Mutagenicity

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations. Two in vivo mammalian micronucleus tests and a Drosophila test were negative. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Thus, there is no concern in relation to exposure of man via the environment.

Conclusion: ii

Carcinogenicity

Tris(2-chloroethyl)phosphate induces the formation of benign and malignant tumors at various sites in experimental animals. Renal tubule cell neoplasms were found in rats and mice each in both sexes. At present the mechanism of tumor development in the kidneys of tris(2-chloroethyl)phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that tris(2-chloroethyl) phosphate appears to have no genotoxic potential. Thus it is likely that the tumor formation in the kidney

would be mediated by non-genotoxic (epigenetic) mechanisms. A species specific mechanism of tumor formation in the kidney was not identified.

At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in formation of kidney tumours (see discussion in chapter 4.1.2.8). It seems possible, that any of the adverse effects described in the TCEP studies on chronic toxicity might be responsible for the induction of tumour development.

Although it is considered likely that tumour formation especially in the kidney would be mediated by a non-genotoxic (epigenetic) mechanism, a threshold level for carcinogenicity has not been established due to the lack of relevant data. Renal tumors in male and female F344/N rats, in male B6C3F1 mice and in male Scl:ddY mice may be induced secondary via cytotoxicity followed by cell proliferation. However, a NOAEL for the cytotoxic effects and also for a cell proliferation mechanism could not be derived.

The scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP have to be recognized. However, one could imagine that any of the adverse effects described in the studies on chronic toxicity of TCEP might be responsible for the induction of tumour development. Given this, the LOAEL for kidney lesions obtained in Scl:ddY mice after feeding of 12 mg/kg bw/d in the diet for 18 months might be used as a surrogate LOAEL for risk assessment purposes on carcinogenicity using a MOS approach. Thus, it is therefore considered justified to perform the risk assessment of man via the environment on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

Local scenario

The calculated total daily intake 0.0058 mg/kg bw/d is compared with an oral LOAEL of 12 mg/kg bw/d (kidney lesions). The margin of safety of 2069 is judged to be sufficient taking into account the assumptions used in derivation of the LOAEL. Thus, the substance is considered to be of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The regional total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety of about 10^6 is judged to be sufficient. Thus, the substance is no concern for tumor formation in relation to regional exposure via the environment.

Conclusion: ii

Toxicity for reproduction

- Fertility

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. Reproductive failure was observed at daily doses of 700 mg/kg bw (cf. 4.1.3.3).

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty.

- Overall confidence in the database

There are no reasons to assume no confidence.

- Intra- and interspecies variation

The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice.

- Differences in exposure (route, duration, frequency and pattern)

The calculated total daily doses were compared with an oral NOAEL.

There are no reasons to assume that special concern can be derived from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenario:

Man exposed indirectly via the environment

Local scenario

The total daily intake was calculated to be 0.0058 mg/kg bw/d. The margin of safety of about 30000 is judged to be sufficient. Thus, the substance is of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety in the range of 10^7 is judged to be sufficient for regional indirect exposure.

Conclusion: ii

- Developmental toxicity

Teratogenic effects of tris(2-chloroethyl) phosphate were investigated up to doses of 200 mg/kg bw/d. No embryo-/fetotoxic or specific teratogenic properties were observed even at maternally toxic doses (NOAEL/maternal toxicity = 100 mg/kg bw/d). Therefore, developmental toxicity is not considered to be of concern in relation to indirect exposure via the environment

Conclusion: ii

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

Tris(2-chloroethyl)phosphate is not explosive.

4.2.2.2 Flammability

Tris(2-chloroethyl)phosphate is not flammable.

4.2.2.3 Oxidising potential

Due to its chemical structure, Tris(2-chloroethyl)phosphate is not expected to possess any oxidizing properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Not applicable

4.2.3.2 Consumers

Not applicable

5 CONCLUSIONS / RESULTS

Overall results of the risk assessment:

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

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

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

Summary of conclusions:

Environment

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

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

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

ad ii)

This conclusion applies to all life cycle steps to all environmental compartments, to the function of waste water treatment plants and to secondary poisoning via the food chain.

TCEP does not meet the PBT criteria.

A potential risk in future cannot be excluded if production and/or use volumes were to increase as a consequence of actions on other flame retardants. Therefore, it is recommended to monitor that the downtrend in use of TCEP is not reversed in future.

Human Health

Consumer

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

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

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

Ad iii) Risk reduction measures are required for babies with respect to the scenario sucking on toys taking into consideration the carcinogenic properties of the substance and the effects after repeated oral administration.

Workers

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

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

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

ad iii)

For TCEP three occupational exposure scenarios are evaluated. TCEP is produced (scenario 1) and is used for the production of formulations (scenario 2). The use of TCEP-containing formulations (scenario 3) includes spray application (scenario 3a) and applications without formation of aerosols (scenario 3b). The overall result of risk assessment indicates that current exposure levels (inhalation and dermal contact) are too high for all occupational exposure scenarios.

From the toxicological point of view, concern mainly derives from the carcinogenic properties of TCEP. In addition, chronic toxicity and partly fertility impairment give reason for concern.

Measures selected for risk reduction should be able to substantially reduce TCEP exposure of workers. Special emphasis should be given to the “spray application” scenario (dermal contact and inhalation).

With respect to risk assessment for carcinogenicity inhalation exposure at the workplace should be reduced to a level of 0.2 mg/m^3 or below. It is recommended to establish an occupational exposure limit for TCEP.

Concerning skin contact, exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Human exposed via the environment

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

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

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

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

6 REFERENCES

Akzo Chemicals (1990a). LSR rept. 90/AKL 023/0230 (unpubl.).

Akzo Chemicals (1990b). LSR rept. 90/AKL 021/0161 (unpubl.).

Akzo Chemicals (1990c). LSR rept. 89/AKL 022/1063 (unpubl.).

Akzo Chemicals (1992). CRL rept. F 92016 (amendment: 1995) (unpubl.).

Akzo Chemicals (1993). Initial Hazard Assessment and Hazard Profile of Fyrol Flame Retardants. R.J. van Wijk, M.G.J. Geurts, C.G. van Ginkel; CRL F93021, 19.01.1993.

Akzo Nobel (1996). Questionnaire on exposure estimation.

Akzo Nobel (2000c). Boerdijk GB., Polymer Chemicals, Safety-Detail Report, Determination of the vapour pressure of Fyrol CEF, measured with vacuum DSC, July 6, 2000.

Akzo Nobel (2000b). O Boen Ho. Memorandum, Polymer Chemicals, Laboratory Deventer, June 15, 2000.

Akzo Nobel (2000a). Peters BFM. Polymer Chemicals, Safety-Detail Report, Differential Scanning Calorimetry Investigation of tri(chloroethyl)phosphate (Fyrol CEF), 10-05-2000

Akzo Nobel (2002). Letter to BAuA, May 2002.

Akzo Nobel (2004). Letter to BAuA, July 2004.

ARGE (2000). Ausgewählte organische Spurenverunreinigungen in der Elbe und Elbenebenflüssen im Zeitraum 1994-1999. Arbeitsgemeinschaft für die Reinhaltung der Elbe. Hamburg. Edited by Fooken C and Stachel B.

ARW (1995). Zur Belastung von Rhein, Main und Rheinuferfiltrat mit Chloralkylphosphaten, 52. Bericht 1995.

Aston LS, Noda J, Seiber JN, and Reece CA (1996). Organophosphate flame retardants in needles of *Pinus ponderosa* in the Sierra Nevada Foothills. Bull Environ. Contam. Toxicol. **57**, 859-866.

AUH (1995). Arbeitskreis Umwelthygiene der Arbeitsgemeinschaft der leitenden Medizinalbeamtinnen und -beamten der Länder: Standards zur Expositionsabschätzung. Behörde für Arbeit, Gesundheit und Soziales, Hamburg.

BAU (1994). Neue Stoffe am Arbeitsplatz: Ein Bewertungskonzept, Amtliche Mitteilungen der Bundesanstalt für Arbeitsschutz, Sonderdruck März 1994, Dortmund.

Baumann W and Muth A (1997). Farben und Lacke 1 - Daten und Fakten zum Umweltschutz, Springer Verlag, Berlin.

Bayer AG (1986). DIN Safety Data Sheet.

Bayer AG (1991). Safety Data Sheet.

BBA (1991). (Biologische Bundesanstalt für Land- und Forstwirtschaft) Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln (Nr. VI, 23-2.1.8): Auswirkungen von Pflanzenschutzmitteln auf Imagines von *Poecilus cupreus* L. als Vertreter der Familie Carabidae (= Laufkäfer) im Laboratorium.

BBA (1992). (Biologische Bundesanstalt für Land- und Forstwirtschaft) Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln (Nr. VI, 23-2.1.9): Auswirkungen von Pflanzenschutzmitteln auf Spinnen der Gattung *Pardosa* (Araneae, Lycosidae) im Laboratorium (draft).

Becker K, Seiwert M, Kaus S, Krause C, Schulz C and Seifert B. (1998). German Environmental Survey 1998 (GerES III): Pesticides and other pollutants in house dust. In: INDOOR AIR '02. Proceedings of the 9th International Conference on Indoor Air Quality and Climate Vol. IV. Monterey, CA, USA, June 30-July 5, 2002, p. 883-887.

Belke M, Pnick I, Evtimova R, Langenstraßen R and Behrendt G (2003). Closed Loop of Polyurethane Flexible Slabstock Foam Wastes by a Chemical Process. PFA Conference, Montreal, Canada, October 2003

Bester K, Grundmann A, Meyer J and Spiteller M (2003). Organophophate flame retardants in surface waters. Poster presentation at SETAC Europe 13th Annual Meeting, Hamburg, Germany.

BgVV (1997). Case register of Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Case 1061/97.

Biethan U et al. (1979). Lacke und Lösemittel, Verlag Chemie, Weinheim.

Bissell RE (1977). A Novel synthesis of 1,4-Diarylpiperazines. J. Heterocycl. Chem., **14**, 535-536

Blume and Ahlsdorf (1993). Ecotoxicology and Environmental Safety **26**, 313-332.

Brown et al. (1975). Research program on hazard priority ranking of manufactured chemicals, Phase II - Final Report, Stanford Research Institute, PB-263164.

Bruckert HJ and Schoene K (1990). Austritt von TRIS(2-Chlorethyl)Phosphat aus Bezugsstoffen für Sitzpolster. Fraunhofer Institut für Umwelthygiene und Ökotoxikologie; unpublished.

Burka LT, Sanders JM, Herr DW and Matthews HB (1991). Metabolism of tris(2-chloroethyl)phosphate in rats and mice. Drug Metab. Dispos.; **19**: 443-447.

Burkhardt G et al. (1992). Plastics, processing. In: Ullmann´s Encyclopedia of Industrial Chemistry, 5th Ed., VCH, Weinheim.

Bürgi D (2002). Phosphorus-based flame retardants in indoor air. Bundesamt für Gesundheit, Bern, Schweiz, Rep. No 02.59 (1).

Bürkholz A, Widder J, Müller W and Dislich M (1976). Primäre Tröpfchengrößenverteilung und atembarer Feinanteil beim Lackspritzen durch Druckluftzerstäuben, Farbe und Lack **8**, S. 693.

Calabrese EJ, Barnes R, Stanek EJ, Pastides H, Gilbert CE, Venemann P and Wang X (1989). How much soil do young children ingest: An epidemiologic study. Regul. Toxicol. Pharmacol. **10,** 123-137.

CEPE (2002). Survey by European Council of Paint, Printing Inks and Artists' Colours Industry, 20.06.2002, provided by CEFIC, 26.05.2003.

CEPE (2004). Survey by European Council of Paint, Printing Inks and Artists' Colours Industry, 2.08.2004

Chapman DE, Michener SR and Powis G (1991). Metabolism of the flame retardent plasticizer tris(2-chloroethyl)phosphate by human and rat liver preparations. Fundam. Appl. Toxicol. ; **17,** 215-224.

Chadwick M, Hayes D, Schepis JP and Nomeir AA (1989). Disposition of 14C-Tris(2-chloroethyl)phosphate (TRCP) in male Fischer-344 rats after oral administration. Toxicologist, **9**, R344.

Claude A (1958). Adenocarcinome renal endemique chez une souche pure de souris; son effet sur la croissance. Rev. Eur. Etud. Clin. Biol. **3,** 261-262.

Cohen SM, and Friedell GH (1982). Neoplasms of the urinary system. In: Foster HL, Small JD, Fox JG (eds), the mouse in biomedical research. Vol.IV: Experimental biology and oncology. Academic Press, New York, 439-463.

Courtaulds Chemicals (1996). Questionnaire on exposure estimation.

Crook JW and Haggis GA (1969). Some Aspects of the Behaviour of Halogen Containing Phosphates and Phosphonates in Urethane Foams. J. Cellular Plastics **15**, 119-122

Danish EPA (2004). Survey and risk assessment of perfume and flavours in toys and child care products. Survey of chemical substances in consumer products no. Xx. Draft (in Danish).

DECOS (1995). (Dutch Expert Committee for Occupational Standards) Calculating cancer risk. No 1995/06 WGD, The Hague.

Delogu B (2000). Understanding the Precautionary Principle. Presentation at the International Conference on Chemical Control Regulations as representative of EU DG SANCO, 10/12 May 2000, Documentation ChemCon 2000, Austrian Federal Economic Chamber, Salzburg, Austria.

Dorsey, L.C. (1997). Report of the FIFRA Scientific Advisory Panel (SAP) Meeting on cholinesterase inhibition policy. June 1997 (http:\\www.epa.gov/opp00001/SAP/archive/june/inhibit.htm)

Dybing E, Sanner T, Roelfzema H, Kroese D and Tennant RW (1997). T25: A simplified carcinogenic potency index: descriptions of the systemic and study of correlations between

carcinogenic potency and species/site specificity and mutagenicity. Pharmacol. Toxicol. **80**, 272-279.

EA (2003). Emission Scenario Document – Chemicals used in the Coatings Industry: Paints, Lacquers and Varnishes, May 2003, Environment Agency UK.

EC (1991). Directive 91/414: Council Directive of 15 July 1991 concerning the placing of plant protectin products on the market. Commission of the European Communities, L 230.

EC (2003a). Technical Guidance Document (TGD) in support of Commision Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commision Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances.

EC (2003b). European Union Risk Assessment Report "2,2-Bis(chloromethyl)trimethylene bis[bis(2-chloroethyl)phophate]" (V6), Draft February 2003.

EC (2005). European Union Risk Assessment Report "Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP), Draft August 2005.

EC (2006a). European Union Risk Assessment Report "Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP), Draft October 2006.

EC (2006b). European Union Risk Assessment Report "2,2-Bis(chloromethyl)trimethylene bis[bis(2-chloroethyl)phophate]" (V6), Draft October 2006.

ECB (2000). TGD Revision, Chap. 3.5 Toxicokinetics, Draft October 10, 2000, Tgd_h_eft_dr_1_annexB_cdh.

Eiben R (2001). Frequency and time of spontaneous tumors found in B6C3F1 mice oncogenicity studies over 10 years. Exp. Toxic. Pathol. **53**, 399-408.

Eldefrawi AT, Mansour NA, Brattsten LB, Ahrens VD and Lisk DJ (1977). Further toxicological studies with commercial and candidate flame retardant chemicals. Part II. Bull. Environ. Contam. Toxicol. **17**, 720-726.

EPA (1997). (Environmental Protection Agency) Exposure Factors Handbook. Update to Exposure Factors Handbook EPA/600/8-89/043 – May 1989. Office of Research and Development, National Center for Environmental Assessment. Vol. I-III. EPA/600/P-95/002Fa, August 1997, Washington, DC.

Environment Agency (2003). TCEP Monitoring data for Midland region 1990-2003. Environment Agency, Wallingford, UK. Letter from 30th July 2003.

Environment Agency (2005), UK. Pers. comm. 7th September 2005.

Environment Agency (2006), UK. TCEP Groundwater monitoring data from England 2004-2005. Email by Jane Caley from 7th February 2006.

EU RAR TCPP (2006). Risk Assessment Tris(2-chloro-1-methylethyl) phosphate (TCPP) Draft Human Health Report, January 2006.

Eustis SL, Hailey JR, Boorman GA and Haseman J (1994). The utility of multiple-section sampling in histopathological evaluation of the kidney for carcinogenicity studies. Toxicol. Pathol **22,** 457-472.

Finley BL, Proctor D, Scott P, Harrington N, Paustenbach D and Price P (1994). Recommended distributions for exposure factors frequently used in health risk assessments. Risk. Anal. **14,** 533-553.

Freeman, N., Adgate, J. (2003): Estimates of dust loading on the hands of children and dust ingestion. ISEA 13th Annual Conference, Stresa, Italy, 2003

Fries E and Püttmann W (2001). Occurrence of organophosphate esters in surface water and ground water in Germany. J. Environ. Monit. **3**, 621-626.

Fries E and Püttmann W (2003). Monitoring of the three organophosphate esters TBP, TCEP and TBEP in river water and ground water (Oder, Germany). J. Environ. Monit. **5**, 346-352.

Frith CH (1986). Incidence of neoplastic and nonneoplastic lesions in several strains of mice, Little Rock, AR, Toxicology Pathology Associates, p 50.

Galloway SM, Armstrong MJ, Reuben C, Colman, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA and Anderson B (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Eva-luations of 108 chemicals. Environ. Mol. Mutagen., **10,** 1-175.

Galassi S (1991). Organophosphorus compounds in the River Po and in the Northern Adriatic. Toxicol. Environ. Chem., **31**-**32**, 291-296.

GDCh (1987). BUA report No. 20, Tris(2-chlorethyl)-phosphat, VCH, Weinheim.

Grasso P (1987a). Experimental liver tumors in animals. In: Williams R and Johnson P-J (Eds), Clinical Gastroenterology – Liver Tumors. Balliere Tyndall, London. Philadelphia, p. 183.

Grasso P (1987b). Persistent organ damage and cancer production in rats and mice. Mechanisms and models in toxicology. Arch. Toxicol. Suppl. **11**, 75-87.

Gulati DK and Russell S (1985). Tris(2-chlorethyl)phosphate: Sperm Morphology Vaginal Cytology in Rodents. Report (Technical Protocol), Contract No N01-ES-3-5026, Study No SMVCE-82-001, Environ. Health Res. and Test. Inc.

Gulati DK, Hommel Barnes L, Chapin RE and Heindel J (1991). Tris(2-chlorethyl)phosphate Reproduction and Fertility Assessment in Swiss CD-1 Mice when Administered via Gavage. Final Report, Contract No NTP-NO1-ES-65142, Study No NTP-89-RACB-070; NTIS/PB92-129170.

Hansen D, Volland G, Krause G and Zoeltzer D (2000). Determination and occurrence of organophosphorous compounds (POC) in house dust and indoor air. Otto-Graf-Journal **11,** 201 -209.

Hansen M K, Larsen M and Cohr K-H (1987). “Waterborne paints”, Scand. J. Work Environ. Health, **13**, 473 – 485.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, Mackenzie KM, Piccirillo VJ and Smith KN (1987). Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog. Carcinog. Mutagen. **7,** 29-48.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutag., **1,** 3-142.

Hazelton Europe (1994a). Physical Chemistry Section, Solubility of TCEP in water, 18 April 1994.

Hazelton Europe (1994b). Physical Chemistry Section, Test: Partition coefficient, 20 April 1994.

Herr DW, Sanders JM and Matthews HB (1991). Brain distribution and fate of tris(2-chloroethyl)phosphate in Fischer 344 rats. Drug Metabol. Dispos., **19**, 436-442.

Hoechst (1994). Technical information.

Hoechst AG (1978). Rept. 6/78 (unpubl.).

Hoechst AG (1985). Rept. W 84-537 (unpubl.).

Hoechst AG (1988). Rept. OEK 88/082 E (unpubl.).

Hoechst AG (1989). Rept. HOE CG 0101 OA ZD00 001; 9717 (unpubl.).

Hoechst AG (1994). EU Safety Data Sheet Hostaflam OP 810.

Hoechst AG (1988a). Pharma Forschung Toxikologie und Pathologie. Tris(2-chlorethyl)-phosphat. Prüfung auf Hautreizung am Kaninchen. Unpublished report Nr. 88.0638, 11.5.1988.

Hoechst AG (1988b). Pharma Forschung Toxikologie und Pathologie. Tris(2-chlorethyl)-phosphat. Prüfung auf Augenreizung am Kaninchen. Unpublished report Nr. 88.0709, 26.5.1988.

Huntingdon Research Centre (1990). Acute oral toxicity to rats of tris(2-chloroethyl)phosphate. Unpublished report No. 891299D/CLD 48/AC, 25.5.1990.

Huntingdon Research Centre (1991b). Eye irritation to the rabbit of tris-2-chloro-ethyl phosphate. Unpublished report No. 91210D/CLD 60/SE, 17.7.1991.

Huntingdon Research Centre (1991a). Skin irritation to the rabbit of tris 2-chloro-ethyl phosphate. Unpublished report No. 901196D/CLD 57/SE, 22.6.1991.

Ihara M, Tajima M, Yamate J and Shibuya K (1994). Morphology of spontaneous Harderian gland tumors in aged B6C3F1 mice. J. Vet. Med. Sci. 56, 775-8.

Ingerowski G, Friedle A and Thumulla J (2001). Chlorinated ethyl and isopropyl phosphoric acid triesters in the indoor environment - An inter-laboratory study. Indoor Air, **11**, 145 - 149.

IPCS (1998). Flame Retardants: Tris(chloropropyl)Phosphate and Tris(2-chloroethyl) Phosphate. Environmental Health Criteria 209. International Programme on Chemical Safety, WHO, Geneva.

IRI (1993). Project No. 753060 Tris(2-chloroethyl)phosphate micronucleus test in bone marrow of CD-1 mice Report No. 9699. **In**: Inveresk Research International, 1-42.

JMPR (1998). Report of a consultation on interpretation of inhibition of acetylcholinesterase activity. Geneva, 8-9 January 1998.

Kanisawa M and Shigetoshi S (1978). Induction of renal and hepatic tumors in mice by ochratoxin A, a mycotoxin. Gann, **69**, 599-600.

Kawashima K, Tanaka S, Nakaura S, Nagao S, Endo T, Onada K, Takanaka A and Omori Y (1983). Effect of Oral Administration of Tris(2-chloroethyl)phosphate to Pregnant rats on Prenatal and Postnatal Developments. Eisli Shikenjo Hokuku, **101,** 655-61.

Kersten W and Reich T (2003). Schwer flüchtige organische Umweltchemikalien in Hamburger Hausstäuben. Gefahrstoffe - Reinhaltung der Luft **63,** 85 - 91.

Kirk-Othmer (1993). Encyclopedia of Chemical Technology, 4th Ed., Vol. 10.

Knepper TP and Karrenbrock F (1996). Zur Belastung von Main, Rhein und Rheinuferfiltrat mit Chloralkylphosphaten. ARW Jahresbericht 1995.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB and Buxton HT (2002). Pharmaceutical, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A National Reconnaissance. Env. Sci. Technol. , **36**, 1202-1211.

Kühn R, Pattard M and Pernak K-D (1989). WaBoLu Res. rept. 106 03 052/01 (UBA): Evaluation of the environmental hazard in aquatic systems: Damaging effects of selected environmental chemicals - Extended testing of toxicity for *Daphnia magna* as a basis of water quality standards.

Laniewski K, Borén H and Grimvall A (1998). Identification of volatile and extractable chloroorganics in rain and snow. Environ. Sci. Technol., **32**, 3935-3940.

Le Bel GL and Williams DT (1983). Determination of organic phosphate triesters in human adipose tissue. J. Assoc. Off. Anal. Chem., **66,** 691-699.

Levchik S, Luda MP, Bracco P, Nada P and Costa L (2004). Discoloration in Fire-Retardant Flexible Polyurethane Foams. API Plastics Conference, Las Vegas, October 18-20, 2004, pp. 46-52

Loeb HA and Kelly WH (1963). U.S. Fish. Wildl. Serv., Sp. Sci. Rep.-Fish. No. 471, Washington, D.C.: 124 p.; cited in AQUIRE (29.01.1992).

Ludvik G F and Decker GC (1947). Toxicity of certain esters of phosphorus acids to aphids. J. Econ. Entom., **40,** 97-100.

Marklund A, Andersson B and Haglund P (2003). Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere, **53**, 1137-1146.

Matthews HB, Dixon D, Herr DW and Tilson H (1990). Subchronic toxicity studies indicate that tris(2-chloroethyl)phosphate administration results in lesions in the rat hippocampus. Toxicol. Industr. Health, **6,** 1-15.

Matthews HB, Eustis SL and Haseman J (1993). Toxicity and Carcinogenicity of Chronic Exposure to Tris(2-chloroethyl)phosphate. Fundam. Appl. Toxicol., **20**, 477-485.

Meyer J and Bester K (2004): Organophosphate flame retardants and plasticizers in wasterwater treatment plants. J. Environ. Monit., **6**, 599 – 605.

Metzger JW and Möhle E (2001).: Flammschutzmittel in Oberflächengewässern, Grundwässern und Abwässern – Eintragspfade und Gehalte. Förderkennzeichen: BWB 99012.

Minegish KI, Kurebayashi H and Nambaru S (1988). Comparative studies on absorption, distribution and excretion of flame retardants halogenated alkyl phosphate in rats. Eisei Kagaku, **34,** 102-114.

MITI (1992). Biodegradation and bioaccumulation data of existing chemicals based on the CSCL, Japan. Chemical Ind. Ecol.-Toxicology a. Inform. Center.

Mobil (1983). Mobil Environmental and Health Science Laboratory, Princeton, New Jersey, USA. Delayed contact hypersensitivity study in guinea pigs (Buehler test) of Antiblaze 100 (AB-100) Bericht, Study No. 33076 (1983d) NTIS/OTS 0511776. Translation from the Toxicological Evaluation No. 33: Tris(2-chlorethyl)-phosphat (12/95). BG Chemie, Heidelberg, 1995, p. 18

Morrissey RE, Schwetz BA, Lamb JC, Ross MD, Teague JL and Morris RW (1988). Evaluation of Rodent Sperm, Vaginal Cytology, and Reproductive Organ Weight Data from National Toxicology Program 13-Week Studies. Fundam. Appl. Toxicol., **11,** 343-358.

MUNLV NRW (2003): Untersuchungen zum Eintrag und zur Elimination von gefährlichen Stoffen in kommunalen Kläranlagen. Part 1. Final Report, September 2003. Research project funded by Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes Nordrhein-Westfalen (MUNLV NRW)

Nagorka R and Ullrich D (2003). Nachweis von phosphororganischen Flammschutzmitteln im Staubniederschlag und im Schwebstaub: Screening mit GC/NPD. Gefahrstoffe - Reinhaltung der Luft, **63,** 79 - 84.

Nakamura A, Tateno N, Kojima S, Kaniwa M-A and Kawamura T (1979). The mutagenicity of halogenated alkanols and the phosphoric acid esters for Salmonella typhimurium, Mutation Res., **66,** 373-380.

Nakamura K, Kuramoto K, Shibasaki K, Shumiya S and Ohtsubo K (1992). Age-related incidence of spantaneous tumores in SPF C57BL/6 and BDF1 mice. Exp. Anim., 41, 279-285.

Niedersächsisches Landesamt für Ökologie (1997). Orientierende Untersuchungen von Gewässersedimenten auf Nitro-/Polymoschusverbindungen und die Flammschutzmittel TCEP und TCPP.

NO_NL (1999). Guidelines for quantitative risk characterisation of non-threshold carcinogens in the framework of existing chemicals following Council Regulation (EEC) 793/93, Draft 09.08.99, Commission Working Group on the Technical Meetings for Risk Assessment for Existing Substances, NO_NL/01/99.

Noack (1993). Project N° 930818HH (unpubl.).

NTP (1984). National Toxicology Program: Technical report No. 274 Toxicology and carcinogenesis studies of tris(2-ethylhexyl)phosphate (CAS No. 78-42-2) in F344/N rats and B6C3F1 mice (gavage studies)". NIH Publication No. 84-2530. National Institute of Environmental Health Sciences, Research Triangel Park, NC, 178p.

NTP (1985). National Toxicology Program: Toxicology and Carcinogenesis Studies of 2-Chloroethanol (Ethylene Chlorohydrin) (CAS No. 107-07-3) in F344/N Rats and Swiss CD-1 Mice (Dermal Studies). NTP Technical Report No. 275. NIH Publication No. 86-2531. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (1991). National Toxicology Program: Technical report series No. 391. "Toxicology and carcinogeneses studies of tris(2-chloroethyl)phosphate (CAS No. 115-96-8) in F344/N rats and B6C3F1 mice (gavage studies)", US Department of Health and Human Services.

Nyholm N and Kusk KO (2003). Review of two algal toxicity study reports. Prepared for Danish Environmental Protection Agency. January 30th, 2003.

OECD, 2004: OECD Series on Emission Scenario Documents Number 3, Emission Scenario Document on Plastics Additives ENV/JM/MONO/2004(8), June 2004.

Otake T, Yoshinaga I and Yanagisawa Y (2001). Analysis of Organic Esters of Plasticizer in Indoor Air by GC-MS and GC-FPD. Environ. Sci. Technol., **15,** 3099 - 3102.

Otto B (1984). Abschlussbericht Prüfung auf mutagene Wirksamkeit Mikronukleus-Test Prüfsubstanz: Tris(2-chlorethyl)-phosphat, in: (Eds.) Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover.

Pardemann J, Salthammer T, Uhde E and Wensing M (2000). Flame retardants in the indoor environment, Part 1: specification of the problem and results of screening tests. Seppänen O, and Säteri J (Eds.), Proceedings of Healthy Building 2000, Helsinki, Finland 4, 125 - 130.

Percy DH and Jonas AM (1971). Incidence of spontaneous tumors in CD(R)-1 HamM/ICR mice. J. Natl. Cancer Inst., **46**, 1045-1065.

Pers. comm. (2004): Email by Steve Dungey from 9th June 2004, Environment Agency (UK).

Pers. comm. (2006): Email by Jane Caley from 6th January 2006, Environment Agency (UK).

Pers. comm. (2007): Email by Jane Caley from 19th January 2007, Environment Agency (UK).

Prival MJ (1977). Tris(2,3-Dibromopropyl)Phosphate: Mutagenicity of a Widely Used Flame Retardant. Science, **195,** 76-78.

Prösch J, Puchert W and Gluschke M (2000). Occurrence of Chloroalkylphophates in Effluents from Municipal Wastewater Treatment Plants of the German Baltic Sea Catchment Area. Vom Wasser, **95**, 87-96.

Prösch J, Pansch G, and Puchert W (2002). Occurrence of TCEP and TCPP in Bath Lakes as well as House Wells of rural Areas in Mecklenburg-Vorpommern, Germnay. Vom Wasser, **98**, 159-164.

Rabstein LS and Peters RL (1973). Tumors of the kidneys, synovia, exocrine pancreas, and nasal cavity in BALB/cf/Cd mice. J. Natl. Cancer Inst., **51**, 999-1006.

Rabstein LS, Peters RL and Spahn GJ (1973). Spontaneous tumors and pathologic lesions in SWR/J mice. J. Natl. Cancer Inst., **50**, 751-758.

Rennen, MAJ et al., (2002). Prediction of local irritant effects after repeated dermal and respiratory exposure to chemicals. Regul. Toxicol.& Pharmacol., 36, 253-261.

Riepert F (1991). Effects of soil pollutants on Collembola: Determination of the inhibition of reproduction. Revised version 91/10. Biologische Bundesanstalt f. Land- und Forstwirtschaft Berlin.

Römbke J, Bauer C, Brodesser J, Brodsky J, Danneberg G, Heimann D, Renner I & and Schallnaß HJ (1995). Basis for the assessment of the ecotoxicological potential of "existing chemicals" in the terrestrial environment - development of a testing strategy. Batelle Eur. Res. rept. 106 04 103 (UBA), UBA-Texte 53/95 (in German).

Sagunski H, Ingerowski G, Mattulat A and Scheutwinkel M (1997). Tris(2-chlorethyl)phosphat. Exposition und umweltmedizinische Bewertung. Umweltmed. Forsch. Prax. **2,** 185-192.

Sagunski H, Roßkamp E (2002). Richtwerte für die Innenraumluft: Tris(2-chlorethyl)-phosphat. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz, **3**, 300 - 3006.

Sala M, Gu ZG, Moens G and Chouroulinkow I (1982). In vivo and in vitro biological effects of the flame retardants tris(2,3-dibromopropyl) phosphate and tris(2-chlorethyl) orthophosphate. Eur. J. Cancer. Clin. Oncol., **18,** 1337-1344.

Salthammer T and Wensing M (2002). Flame retardants in the indoor environment. Part IV: Classification of experimental data from house dust, indoor air and chamber tests. Levin H (ed.), Proceedings of the 9th International Conference on Indoor Air and Climate - Indoor Air 02, Monterey, CA, 2, 213 - 218.

Sanders JM, Herr DW, Burka LT and Matthews HB (1990). Metabolism of 14C-tris(2-chloroethyl)phosphate (TRCP) in rats and mice. Toxicologist, **10,** R 740.

Sasaki K et al. (1982). Bioconcentration and excretion of phosphoric acid triesters by killifish (Oryzas latipes). Bull. Environ. Contam. Toxicol., **28**, 752-759.

Sasaki K, Suzuki T and Takeda M (1985). Metabolism of phosphoric triesters by goldfish, Carassius auratus. Eisei Kagaku, **31**, 397-404.

Sasaki K, Takeda M and Uchiyama M (1981). Toxicity, absorption and elimination of phosphoric acid triesters by killifish and goldfisch. Bull. Environ. Contam. Toxicol., **27**, 775-782.

Sette, W.F. (1997). The use of data on cholinesterase inhibition for risk assessment of organophosphate and carbamate pesticides. OPP, U.S. EPA prepared draft document for the Scientific Advisory Panel (SAP) review. 30th April 1997.

Shepelskaja NR and Dyschinewitsch NE (1981). Experimental study of the gonadotropic effect of Tris(2-chlorethyl)-phosphat, Gig.Sanit., **6,** 20-21.

Sher SP (1974). Tumors in control mice: literature tabulation. Toxicol. Appl.Pharmacol., **30**, 337-359.

Smyth HF, Carpenter CP and Weil CS (1951). Range-finding toxicity data: List IV. Arch. Ind. Hyg. Occup. Med., **4,** 119-122.

Sprague GL, Sandvik LL, Brookins-Hendricks MJ and Bickford AA (1981). Neurotoxicity of two organophosphorus ester flame retardants in hens. J. Toxicol. Environ. Health, **8**, 507-518.

Stauffer (1974). Chemical Company Western Reasearch Center. Unpublished report T-4781 on the evaluation of the acute inhalation toxicity of Fyrol CEF, 4.5.1974.

Stauffer (1972). Chemical Company Western Reasearch Center. Unpublished toxicology laboratory report T-1275 on Fyrol CEF, 26.10.1972.

Stauffer (1980b). Chemical Company, unpublished data, Report number T-10111, October 1980.

Stauffer (1980a). Chemical Company, unpublished data, Report number T-10111, March 1980.

Stauffer (1979). Chemical Company, unpublished data, Report number T-6438, November 1979.

Stauffer (1976). Report-No. T 5958 Mutagenicity evaluation of fyrol cef (3793-3-2), in: (Eds.) Chemical Company 1976

Stauffer (1978). Report-No. T-6346 Mutagenicity evaluation of fyrol cef (LOT 4736-3-6) in the mouse lymphoma forward mutation assay, in: (Eds.) Chemical Company 1978, 1-9.

Stauffer (1979). Report-No. T-6540 Evaluation of fyrol cef (Lot #4808-3-5) in the unscheduled DNA synthesis in human WI-38 cells assay, in: (Eds.) Chemical Company 1979.

Stoye et al. (1998). Paints and Coatings – Production Technology. In: Ullmann's Encylopedia of Industrial Chemistry, Sixth Ed., Edition Release, Wiley VCH, Weinheim.

Swedish Product Register 1996.

Takada K, Yasuhara K, Nakaji Y, Yoshimoto H, Momma J and Kurokowa Y (1989). Carcinogenicity study of tris(2-chloroethyl)phosphate in ddY mice. J. Toxicol. Pathol., **2,** 213-222.

Tamano S, Hagiwara A, Shibata M-A, Kurata Y, Fukushima S and Ito N (1988). Spontaneous tumors in aging (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice. Toxicol. Pathol., 16, 321-326.

Tarone RE, Chu KC and Ward JM (1980). Variability in rates of some common naturally occurring tumors in F344 rats and B6C3F1 mice. J. Natl. Cancer Inst., **66**, 1175-1181.

Tomatis l, Turusov V, Day N and Charles RT (1972). The effect of long term exposure to DDT on CF-1 mice. Int. J. Cancer, **10**, 489-506.

Tsuji S, Tonogai Y, Ito Y and Kano S (1986). Effect of rearing temperatures on the toxicity of various environmental pollutants for killifish (Oryzias latipes). Eisei Kagaku, **32**, 46-53.

Turusov VS, Deringer MK, Dunn TB and Stewart HL (1973). Malignant liver tumor in mice resembling human hepatoblastoma. J. Natl. Cancer Inst., **51**, 1689-1695.

Ulfarson U, Alexandersson R, Dahlqvist M, Bergstöm B and Scullman J (1992). "Temporary health effects from exposure to water-borne paints", Scand. J. Work Environ. Health, **18**, 376 - 387.

Ulsamer AG, Osterberg RE and McLaughlin J (1980). Flameretardant chemicals in textiles. Clin. Toxicol., **17,** 101-131.

Vogel EW and Nivard MJM (1993). Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination., Mutagenesis, **8,** 57-81.

Ward JM, Goodman DG, Squire RA, Chu KC and Linhart MS (1979). Neoplastic and nonneoplastic lesions in aging [(C57BL/6NXC3H/HeN)F1 (B6C3F1)] mice. J. Natl. Cancer Inst., **63**, 849-854.

Weidlich U and Gmehling J (1986). Expositionsabschätzung - Ein Methodenvergleich mit Hinweisen für die praktiosche Anwendung, Fb 488, Schriftenreihe der Bundesanstalt für Arbeitsschutz, Wirtschaftsverlag NW, Bremerhaven.

Wensing M, Pardemann J and Schwampe W (2003). Flame retardants in the indoor environment. Part V: Measurement and exposure evaluation of organophosphate esters from automobile interiors. Fraunhofer Institut für Holzforschung, Braunschweig, and TÜV Nord Umweltschutz, Hamburg, Germany. Healthy Buildings 2003 Conference www.hb2003.org (7-11 December 2003, Singapore).

Weisser (1992). Untersuchungen zur Belastung kommunaler Klärschlämme durch organische Schadstoffe, BMFT-Forschungsvorhaben 02 WS 464/8, Schriftenreihe des Instituts für Siedlungswasserwirtschaft, Universität Karlsruhe, Band 63, 1992.

WHO (1990). Environmental Health Criteria 104: Principles for the toxicological assessment of pesticide residues in foods. WHO, Geneva, pp. 63-65

Yamamoto H, Ohno Y, Nakamori K, Okuyama T, Imai S and Tsuburu Y. (1982). Chronic toxicity and carcinogenicity test of 1,2,4-trichlorobenzene on mice by dermal painting. J. Nara Med. Ass. (Nara Igaku Zasshi), **33**, 132-145.

Yoshioka Y and Ose Y (1993). A quantitative structure-activity study and ecotoxicological risk quotient for the protection from chemical pollution. Environ. Toxicol. Water Quality, **8**, 87-101.

Yoshioka Y, Ose Y and Sato T (1986). Correlation of the five test methods to assess chemical toxicity and relation to physical properties. Ecotox. Environ. Safety, **12**, 15-21.

Appendix I

Distribution and fate

Distribution and Fate

d := Tag

Substance: TCEP

melting point: $MP := 219 \cdot K$

vapour pressure: $VP := 13.33 \cdot Pa$

water solubility: $SOL := 7820 \cdot mg \cdot l^{-1}$

part. coefficient octanol/water: $LOGP_{OW} := 1.78$

molecular weight: $MOLW := 0.285 \cdot kg \cdot Mol^{-1}$

gas constant: $R := 8.3143 \cdot J \cdot Mol^{-1} \cdot K^{-1}$

temperature: $T := 285 \cdot K$

conc. of suspended matter in the river: $SUSP_{water} := 15 \cdot mg \cdot l^{-1}$

density of the solid phase: $RHO_{solid} := 2500 \cdot kg \cdot m^{-3}$

volume fraction water in susp. matter: $Fwater_{susp} := 0.9$

volume fraction solids in susp.matter: $Fsolid_{susp} := 0.1$

volume fraction of water in sediment: $Fwater_{sed} := 0.8$

volume fraction of solids in sediment: $Fsolid_{sed} := 0.2$

volume fraction of air in soil: $Fair_{soil} := 0.2$

volume fraction of water in soil: $Fwater_{soil} := 0.2$

volume fraction of solids in soil: $Fsolid_{soil} := 0.6$

aerobic fraction of the sediment comp.: $Faer_{sed} := 0.1$

product of CONjunge and $SURF_{air}$: $product := 10^{-4} \cdot Pa$

distribution air/water: Henry-constant

$$HENRY := \frac{VP \cdot MOLW}{SOL} \qquad HENRY = 0.486 Pa \cdot m^3 \cdot Mol^{-1}$$

$$\log\left(\frac{HENRY}{Pa \cdot m^3 \cdot Mol^{-1}}\right) = -0.314$$

$K_{air\_water} := \frac{HENRY}{R \cdot T}$ $K_{air\_water} = 2.0502 \times 10^{-4}$

solid/water-partition ceefficient Kp_{comp} and total compartment/water-partition coefficient K_{comp_water}

$a := 0.49$ (a,b from TGD, p. 539, equ. for phosphates)

$b := 1.17$ $K_{OC} := 10^{a \cdot LOGP_{OW} + b} \cdot l \cdot kg^{-1}$ $K_{OC} = 110.205 l \cdot kg^{-1}$

Suspended matter

$Kp_{susp} := 0.1 \cdot K_{OC}$ $Kp_{susp} = 11.02 l \cdot kg^{-1}$

$K_{susp\_water} := Fwater_{susp} + Fsolid_{susp} \cdot Kp_{susp} \cdot RHO_{solid}$ $K_{susp\_water} = 3.655$

factor for the calculation of $Clocal_{water}$:

$faktor := 1 + Kp_{susp} \cdot SUSP_{water}$ $faktor = 1$

Sediment

$Kp_{sed} := 0.05 \cdot K_{OC}$ $Kp_{sed} = 5.511 l \cdot kg^{-1}$

$K_{sed\_water} := Fwater_{sed} + Fsolid_{sed} \cdot Kp_{sed} \cdot RHO_{solid}$ $K_{sed\_water} = 3.555$

Soil

$Kp_{soil} := 0.02 \cdot K_{OC}$ $Kp_{soil} = 2.204 l \cdot kg^{-1}$

$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + Fwater_{soil} + Fsolid_{soil} \cdot Kp_{soil} \cdot RHO_{solid}$

$K_{soil\_water} = 3.506$

Sludge (activated sludge)

$K_{p\_sludge} := 0.37 \cdot K_{OC}$ $K_{p\_sludge} = 40.776 l \cdot kg^{-1}$

Raw sewage

$K_{p\_sewage} := 0.30 \cdot K_{OC}$ $K_{p\_sewage} = 33.061 l \cdot kg^{-1}$

Elimination in STPs

rate constant in STP: k = 0 d^{-1}

elimination P = f (k, logpow, logH) = 2.3 %

fraction directed to surface water $Fstp_{water}$ = 97.7

biodegradation in different compartments

surface water

$kbio_{water} := 0 \cdot d^{-1}$ (cTGD, table 5)

soil

$DT50bio_{soil} := 1 \cdot 10^{6} \cdot d$ (cTGD, table 6)

$kbio_{soil} := \frac{\ln(2)}{DT50bio_{soil}}$ $kbio_{soil} = 2.888 \times 10^{-8} h^{-1}$ $kbio_{soil} = 6.931 \times 10^{-7} d^{-1}$

sediment

$kbio_{sed} := \frac{\ln(2)}{DT50bio_{soil}} \cdot Faer_{sed}$ $kbio_{sed} = 2.888 \times 10^{-9} h^{-1}$

degradation in surface waters

$khydr_{water} := 1.74 10^{-4} \cdot d^{-1}$ (t 1/2 = 3980 d)

$kphoto_{water} := 1 \cdot 10^{-10} \cdot d^{-1}$

$kdeg_{water} := khydr_{water} + kphoto_{water} + kbio_{water}$

$kdeg_{water} = 1.74 \times 10^{-4} d^{-1}$ $kdeg_{water} = 7.25 \times 10^{-6} h^{-1}$

Atmosphere

calculation of CONjunge * SURFaer for the OPS-model

$$VPL := \frac{VP}{\exp\left[6.79 \cdot \left(1 - \frac{MP}{285 \cdot K}\right)\right]} \qquad VP := \text{wenn}(MP > 285 \cdot K, VPL, VP) \qquad VP = 13.33\,Pa$$

$$Fass_{aer} := \frac{product}{VP + product} \qquad Fass_{aer} = 7.502 \times 10^{-6}$$

degradation in the atmosphere

$kdeg_{air}$ = 3.96 * 10^{-2} h^{-1} (see AOP-calculation)

Appendix II

Continental and regional exposure

SimpleBox2.0a - Berechnung regionaler + kontinentaler PEC's

- Anpassung an TGD (1996) / EUSES 1.00: Michael Feibicke (06/98)

INPUT - TCEP

Parameter names acc. SimpleBox20	Unit	Input	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	TCEP	Substance
MOL WEIGHT	[g.mol^{-1}]	285	Molecular weight
MELTING POINT	[° C]	-70	Melting Point
VAPOR PRESSURE(25)	[Pa]	0,00114	Vapour pressure at 25°C
log Kow	[log10]	1,78	Octanol-water partition coefficient
SOLUBILITY(25)	[mg.l^{-1}]	7820	Water solubility
Distribution - Partition coefficients			
- Solids water partitioning (derived from K_{oc})			
Kp(soil)	[l.kg$_d^{-1}$]	2,204	Solids-water partitioning in soil
Kp(sed)	[l.kg$_d^{-1}$]	5,51	Solids-water partitioning in sediment
Kp(susp)	[l.kg$_d^{-1}$]	11,02	Solids-water partitioning in sudpended matter
- Biota-water			
BCF(fish)	[l.kg$_w^{-1}$]	6,5	Biocentration factor for aquatic biota
Degradation and Transfromation rates			
- Characterisation and STP			
PASSreadytest	[y / n]	n	Characterization of biodegradability
- Environmental Total Degradation			
kdeg(air)	[d^{-1}]	9,50E-01	Rate constant for degradation in air
kdeg(water)	[d^{-1}]	1,74E-04	Rate constant for degradation in bulk surface water
kdeg(soil)	[d^{-1}]	6,93E-07	Rate constant for degradation in bulk soil
kdeg(sed)	[d^{-1}]	6,93E-08	Rate constant for degradation in bulk sediment
Sewage treatment (e.g. calculated by SimpleTreat)			
- Continental			
FR(volatstp) [C]	[-]	0,00E+00	Fraction of emission directed to air (STPcont)
FR(effstp) [C]	[-]	9,86E-01	Fraction of emission directed to water (STPcont)
FR(sludgestp) [C]	[-]	1,40E-02	Fraction of emission directed to sludge (STPcont)
- Regional			
FR(volatstp) [R]	[-]	0,00E+00	Fraction of emission directed to air (STPreg)
FR(effstp) [R]	[-]	9,86E-01	Fraction of emission directed to water (STPreg)
FR(sludgestp) [R]	[-]	1,40E-02	Fraction of emission directed to sludge (STPreg)
Release estimation			
- Continental			
Edirect(air) [C]	[t.y^{-1}]	0,6692	Total continental emission to air
STPload [C]	[t.y^{-1}]	1,258	Total continental emission to wastewater
Edirect(water1) [C]	[t.y^{-1}]	6,869	Total continental emission to surface water
Edirect(soil3) [C]	[t.y^{-1}]	0	Total continental emission to industrial soil
Edirect(soil2) [C]	[t.y^{-1}]	0	Total continental emission to agricultural soil
- Regional			
Edirect(air) [R]	[t.y^{-1}]	0,341	Total regional emission to air
STPload [R]	[t.y^{-1}]	1,558	Total regional emission to wastewater
Edirect(water1) [R]	[t.y^{-1}]	0,876	Total regional emission to surface water
Edirect(soil3) [R]	[t.y^{-1}]	0	Total regional emission to industrial soil
Edirect(soil2) [R]	[t.y^{-1}]	0	Total regional emission to agricultural soil

OUTPUT - TCEP

Zur **Neuberechnung der Daten**: ->Extras ->Optionen ->Berechnen -> Datei_berechnen -> F9 drücken, sonst keine komplette Neuberechnung aller Bezüge!!

Parameter names acc. SimpleBox20	Unit	Output	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	TCEP	Substance
Output			
- Continental			
PECsurfacewater (total)	[mg.l^{-1}]	1,18E-05	Continental PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	1,18E-05	Continental PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	5,17E-12	Continental PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	1,23E-06	Continental PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	5,94E-07	Continental PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	1,12E-06	Continental PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	1,12E-06	Continental PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	3,50E-05	Continental PEC in sediment (total)
- Regional			
PECsurfacewater (total)	[mg.l^{-1}]	8,71E-05	Regional PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	8,70E-05	Regional PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	2,27E-10	Regional PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	6,09E-05	Regional PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	2,95E-05	Regional PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	4,91E-05	Regional PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	4,91E-05	Regional PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	2,51E-04	Regional PEC in sediment (total)

Appendix III

Indirect exposure via the environment

INDIRECT EXPOSURE VIA THE ENVIRONMENT

(TGD On New and Existing Chemicals, chapter 2)

Parameter [Unit]	*Symbol*

Definitions (for the use in this document)

definition of the unit 'kg_{bw}' for body weight	$kg_{bw} := 1 \cdot kg$
definition of the unit 'd' for day	$d := 1 \cdot Tag$
	$\mu g := 10^{-3} \cdot mg$
	$scenario := 1 .. 2$
	$local := 1$
	$regional := 2$

Constants

gas - constant R	$R := 8.314 J \cdot K^{-1} \cdot Mol^{-1}$

Defaults

volumefraction air in plant tissue [-]	$Fair_{plant} := 0.3$
volumefraction water in plant tissue [-]	$Fwater_{plant} := 0.65$
volumefraction lipids in plant tissue [-]	$Flipid_{plant} := 0.01$
bulk density of plant tissue [$kg_{wet\ plant} * m_{plant}^{-3}$]	$RHO_{plant} := 700 \cdot kg \cdot m^{-3}$
leaf surface area [m^2]	$AREA_{plant} := 5 \cdot m^2$
conductance (0.001 $m*s^{-1}$) [$m*d^{-1}$]	$g_{plant} := 0.001 \cdot m \cdot s^{-1}$
shoot volume [m^3]	$V_{leaf} := 0.002 \cdot m^3$
transpiration stream [m^3*d^{-1}]	$Q_{transp} := 1 \cdot 10^{-3} \cdot m^3 \cdot d^{-1}$
correction exponent for differences between plant lipids and octanol [-]	$b := 0.95$
growth rate constant for dilution by growth [d^{-1}]	$kgrowth_{plant} := 0.035 d^{-1}$
pseudo-first order rate constant for metabolism in plants [d^{-1}]	$kmetab_{plant} := 0 \cdot d^{-1}$
pseudo-first order rate constant for photolysis in plants [d^{-1}]	$kphoto_{plant} := 0 \cdot d^{-1}$

concentration in meat and milk

daily intake of grass
[$kg_{wetgrass}$*d^{-1}]

$IC_{grass} := 67.6 \cdot kg \cdot d^{-1}$

daily intake of soil
[$kg_{wet\ soil}$*d^{-1}]

$IC_{soil} := 0.46 \cdot kg \cdot d^{-1}$

daily intake of air
[m_{air}^3*d^{-1}]

$IC_{air} := 122 \cdot m^3 \cdot d^{-1}$

daily intake of drinkingwater
[l*d^{-1}]

$IC_{drw} := 55 \cdot l \cdot d^{-1}$

daily intake for human

daily intake for the several pathways
[kg_{chem}*d^{-1}] or [m^3*d^{-1}]

$IH_{drw} := 2 \cdot l \cdot d^{-1}$

$IH_{fish} := 0.115 \cdot kg \cdot d^{-1}$

$IH_{stem} := 1.2 \cdot kg \cdot d^{-1}$

$IH_{root} := 0.384 \cdot kg \cdot d^{-1}$

$IH_{meat} := 0.301 \cdot kg \cdot d^{-1}$

$IH_{milk} := 0.561 \cdot kg \cdot d^{-1}$

$IH_{air} := 20 \cdot m^3 \cdot d^{-1}$

bioavailability through route of intake
[-]

$BIO_{inh} := 0.75$

$BIO_{oral} := 1.0$

average body weight of human
[kg]

$BW := 70 \cdot kg_{bw}$

Input

chemical properties

octanol-water partitioning coefficient [-]	$\log K_{OW} := 1.78$ $K_{OW} := 10^{\log K_{OW}}$
Henry - partitioning coefficient [Pa*m3*mol-1]	$HENRY := 4.155 \cdot 10^{-5} \cdot Pa \cdot m^3 \cdot Mol^{-1}$
air-water partitioning coefficient [-]	$K_{air\_water} := 1.7534 \cdot 10^{-8}$
fraction of the chemical associated with aerosol particles [-]	$F_{ass\_aer} := 0.081$
half-life for biodegration in surface water [d]	$DT_{50\_bio\_water} := 1 \cdot 10^{6} \cdot d$

environmental concentrations

annual average local PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PEClocal_{water\_ann} := 13.59 \cdot \mu g \cdot l^{-1}$
annual average local PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PEClocal_{air\_ann} := 0.0038 \cdot \mu g \cdot m^{-3}$
local PEC in grassland (total), averaged over 180 days [mg_{chem} * kg_{soil}^{-1}]	$PEClocal_{grassland} := 8.66 \cdot \mu g \cdot kg^{-1}$
local PEC in porewater of agriculture soil [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{agr\_soil\_porew} := 18.1 \cdot \mu g \cdot l^{-1}$
local PEC in porewater of grassland [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{grassland\_porew} := 4.18 \cdot \mu g \cdot l^{-1}$
local PEC in groundwater under agriculture soil [mg_{chem} * l_{water}^{-1}]	$PEClocal_{grw} := 18.1 \cdot \mu g \cdot l^{-1}$
regional PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PECregional_{water} := 0.0871 \cdot \mu g \cdot l^{-1}$
regional PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PECregional_{air} := 2.27 \cdot 10^{-10} \cdot mg \cdot m^{-3}$
regional PEC in agriculture soil (total) [mg_{chem}*kg_{soil}^{-1}	$PECregional_{agr\_soil} := 6.09 \cdot 10^{-5} \cdot mg \cdot kg^{-1}$
regional PEC in porewater of agriculture soils [mg_{chem}*l_{water}^{-1}	$PECregional_{agr\_soil\_porew} := 2.95 \cdot 10^{-5} \cdot mg \cdot l^{-1}$

Definition of the concentrations used for indirect exposure

$C_{water_{local}} := PEClocal_{water\_ann}$

$C_{air_{local}} := PEClocal_{air\_ann}$

$C_{grassland_{local}} := PEClocal_{grassland}$

$C_{agr\_porew_{local}} := PEClocal_{agr\_soil\_porew}$

$C_{grass\_porew_{local}} := PEClocal_{grassland\_porew}$

$C_{grw_{local}} := PEClocal_{grw}$

$C_{water_{regional}} := PECregional_{water}$

$C_{air_{regional}} := PECregional_{air}$

$C_{grassland_{regional}} := PECregional_{agr\_soil}$

$C_{agr\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grass\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grw_{regional}} := PECregional_{agr\_soil\_porew}$

bioconcentration in fish

bioconcentration factor for fish
[$m_{water}^3 * kg_{chem}^{-1}$]

$BCF_{fish} := 10^{0.85 \cdot \log K_{OW} - 0.7} \cdot l \cdot kg^{-1}$

modified equation for logKow > 6

$BCF_{fish} := wenn\left[\log K_{OW} > 6, \left(-0.278 \cdot \log K_{OW}^2 + 3.38 \cdot \log K_{OW} - 5.94\right) \cdot l \cdot kg^{-1}, BCF_{fish}\right]$

$C_{fish_{scenario}} := BCF_{fish} \cdot C_{water_{scenario}}$

bioconcentration in plants

$K_{plant\_water} := Fwater_{plant} + Flipid_{plant} \cdot K_{OW}^{b}$

$Croot_{agr\_plant_{scenario}} := \frac{K_{plant\_water} \cdot C_{agr\_porew_{scenario}}}{RHO_{plant}}$

$TSCF := 0.784 \cdot e^{\frac{-\left(\log K_{OW} - 1.78\right)^2}{2.44}}$

remark: for $\log K_{OW}$ out of the range from -0.5 to 4.5
the TSCF is limited by the values for $\log K_{OW}$ = -0.5 resp. 4.5

$TSCF := wenn\left(\log K_{OW} < -0.5, 0.903, TSCF\right)$

$TSCF := wenn\left(\log K_{OW} > 4.5, 0.832, TSCF\right)$

$K_{leaf\_air} := Fair_{plant} + \frac{K_{plant\_water}}{K_{air\_water}}$

$kelim_{plant} := kmetab_{plant} + kphoto_{plant}$

$$\alpha := \frac{AREA_{plant} \cdot g_{plant}}{K_{leaf\_air} V_{leaf}} + kelim_{plant} + kgrowth_{plant}$$

$$\beta agr\_plant_{scenario} := C agr\_porew_{scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C air_{scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C leaf\_crops_{scenario} := \frac{\beta agr\_plant_{scenario}}{\alpha \cdot RHO_{plant}}$$

$$\beta grass\_plant_{scenario} := C grass\_porew_{scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C air_{scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C leaf\_grass_{scenario} := \frac{\beta grass\_plant_{scenario}}{\alpha \cdot RHO_{plant}}$$

purification of drinking water

system may defined dependent from the aerobic biodegradation

$$system := wenn\left(DT_{50\_bio\_water} < 10 \cdot d, 0, 1\right)$$

select a column on dependence from log K_{OW}

$$FIndex := wenn\left(logK_{OW} < 4, 0, wenn\left(logK_{OW} > 5, 2, 1\right)\right)$$

$$Fpur_{logKow} := \begin{pmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{pmatrix}$$

$$Fpur := \frac{Fpur_{logKow_{system, FIndex}}}{wenn\left(HENRY > 100 \cdot Pa \cdot m^3 \cdot Mol^{-1}, 2, 1\right)}$$

$$C drw_{scenario} := wenn\left[C grw_{scenario} > \left(C water_{scenario} \cdot Fpur\right), C grw_{scenario}, C water_{scenario} \cdot Fpur\right]$$

Biotransfer to meat and milk

$$BTF_{meat} := 10^{-7.6 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 1.5 to 6.5
the BTF_{meat} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{meat} := wenn\left(logK_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat}\right)$$

$$BTF_{meat} := wenn\left(logK_{OW} > 6.5, 0.07943 kg^{-1} \cdot d, BTF_{meat}\right)$$

$$C_{meat_{scenario}} := BTF_{meat} \cdot \left(\begin{array}{l} C_{leaf\_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \ldots \\ + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw} \end{array} \right)$$

$$BTF_{milk} := 10^{-8.1+logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 3 to 6.5
the BTF_{milk} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{milk} := wenn\left(logK_{OW} < 3, 7.943 \cdot 10^{-6} \cdot kg^{-1} \cdot d, BTF_{milk}\right)$$

$$BTF_{milk} := wenn\left(logK_{OW} > 6.5, 0.02512 kg^{-1} \cdot d, BTF_{milk}\right)$$

$$C_{milk_{scenario}} := BTF_{milk} \cdot \left(\begin{array}{l} C_{leaf\_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \ldots \\ + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw} \end{array} \right)$$

total daily intake for human

daily dose through intake of several pathways
[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{drw_{scenario}} := \frac{C_{drw_{scenario}} \cdot IH_{drw}}{BW}$$

$$DOSE_{air_{scenario}} := \frac{C_{air_{scenario}} \cdot IH_{air} \cdot BIO_{inh}}{BW \cdot BIO_{oral}}$$

$$DOSE_{stem_{scenario}} := \frac{C_{leaf\_crops_{scenario}} \cdot IH_{stem}}{BW}$$

$$DOSE_{root_{scenario}} := \frac{Croot_{agr\_plant_{scenario}} \cdot IH_{root}}{BW}$$

$$DOSE_{meat_{scenario}} := \frac{C_{meat_{scenario}} \cdot IH_{meat}}{BW}$$

$$DOSE_{milk_{scenario}} := \frac{C_{milk_{scenario}} \cdot IH_{milk}}{BW}$$

$$DOSE_{fish_{scenario}} := \frac{C_{fish_{scenario}} \cdot IH_{fish}}{BW}$$

total daily intake for human

total daily intake for human as sum of each pathway
[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{tot_{scenario}} := DOSE_{drw_{scenario}} + DOSE_{fish_{scenario}} + DOSE_{stem_{scenario}} + DOSE_{root_{scenario}} \ldots + DOSE_{meat_{scenario}} + DOSE_{milk_{scenario}} + DOSE_{air_{scenario}}$$

relative doses of specific different pathway (%)

$$RDOSE_{drw_{scenario}} := \frac{DOSE_{drw_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{air_{scenario}} := \frac{DOSE_{air_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{stem_{scenario}} := \frac{DOSE_{stem_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{root_{scenario}} := \frac{DOSE_{root_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{meat_{scenario}} := \frac{DOSE_{meat_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{milk_{scenario}} := \frac{DOSE_{milk_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{fish_{scenario}} := \frac{DOSE_{fish_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

Results of calculation

$DOSE_{tot_{local}} = 5.842007 \times 10^{-3} \frac{mg}{kg_{bw} \cdot d}$

$DOSE_{tot_{regional}} = 1.110433 \times 10^{-5} \frac{mg}{kg_{bw} \cdot d}$

$RDOSE_{drw_{local}} = 8.852144\%$

$RDOSE_{drw_{regional}} = 22.410813\%$

$RDOSE_{air_{local}} = 0.013938\%$

$RDOSE_{air_{regional}} = 4.380529 \times 10^{-4}\%$

$RDOSE_{stem_{local}} = 85.870726\%$

$RDOSE_{stem_{regional}} = 66.814222\%$

$RDOSE_{root_{local}} = 2.770143\%$

$RDOSE_{root_{regional}} = 2.375282\%$

$RDOSE_{meat_{local}} = 7.832217 \times 10^{-4}\%$

$RDOSE_{meat_{regional}} = 1.997168 \times 10^{-3}\%$

$RDOSE_{milk_{local}} = 7.66065 \times 10^{-3}\%$

$RDOSE_{milk_{regional}} = 0.019534\%$

$RDOSE_{fish_{local}} = 2.484604\%$

$RDOSE_{fish_{regional}} = 8.377713\%$

European Commission

EUR [ECB: click here to insert EUR No.] - European Union Risk Assessment Report [ECB: click here to insert SUBSTANCE NAME, and volume no.]

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Environment and quality of life series

ISBN [ECB: insert ISBN No.]

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The report provides the comprehensive risk assessment of the substance Tris (2-chloroethyl) phosphate. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.
There is no concern identified for the environment from the production and use of the substance.

For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.The human health risk assessment concludes that there is concern for workers with regard to carcinogenicity, repeated dose toxicity and impairment of fertility. For consumers (babies) there is concern for repeated oral exposure. There is no concern for humans exposed via the environment and for human health (physico-chemical properties).