2,4,4-TRIMETHYLPENTENE

CAS No: 25167-70-8
EINECS No: 246-690-9

RISK ASSESSMENT

28.05.2008

Germany

FINAL APPROVED VERSION

The risk assessment of 2,4,4-trimethylpentene has been prepared by Germany on behalf of the European Union.

Contact point:
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Anmeldestelle Chemikaliengesetz (BAuA)
(Federal Institute for Occupational Safety and Health
Notification Unit)
Friedrich-Henkel-Weg 1-25
44149 Dortmund (Germany)
fax: +49(231)9071-2679
e-mail: chemg@baua.bund.de

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

The first draft of the Human Health Section of the Comprehensive Risk Assessment Report of **2,4,4-trimethylpentene**, a substance chosen from the EU 2nd priority list in 1995 was distributed for preliminary written procedure in November 2002.

The Human Health Section was discussed "in-depth" at the Technical Meeting in June 2003 (TM II´03), as "last-visit" at the Technical Meeting in December 2004 (TM IV´04) and was distributed for the final written approval in August 2005.

The first draft of the **Environment Section** of the Comprehensive Risk Assessment Report of 2,4,4-trimethylpentene was distributed for preliminary written procedure in May 2007.

The Environment Section was discussed "in-depth" at TC NES IV´07 (December 2007) and was distributed for the "last-visit written procedure" in January 2008.

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0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 25167-70-8

EINECS No. 246-690-9

IUPAC Name 2,4,4-Trimethylpentene

Overall results of the risk assessment:

(X) 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

Summary of conclusions:

Environment

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

- Releases into the waste water treatment plant for both production sites and for 23 out of 25 processing sites.
- Releases into surface waters during production for both production sites and for 23 out of 25 processing sites.

- Releases into the sediment for both production sites and for 23 out of 25 processing sites.
- Site specific exposure information for 2 sites for refinement of the assessment of non compartment specific effects relevant for the food chain. (probably covered by previous information requests)
- Activated sludge respiration inhibition test (OECD 209)
- Prolonged daphnia magna reproduction test (OECD 211)

Test and exposure information requirements have been agreed at TC NES IV/07.

Conclusion (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.

- Releases into the waste water treatment plant for processing sites A and B.
- Releases into surface waters during production for processing sites A and B.
- Releases into the sediment for processing sites A and B.
- Releases into the atmosphere.
- Releases into the terrestrial compartment.
- Non compartment specific effects relevant for the food chain for one production site and 24 out of 25 processing sites.
- 2,4,4-trimethylpentene does not meet the PBT criteria.

Human Health

Workers

Conclusion (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.

The toxicity profile of 2,4,4-trimethylpentene does not appear very marked. In combination with the dermal and inhalation exposure levels at the workplace no occupational scenarios of concern have been identified.

Consumers

Conclusion (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.

Man exposed indirectly via the environment

Conclusion (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

Identification of the substance

CAS No.: 25167-70-8

EINECS No.: 246-690-9

IUPAC Name: 2,4,4-Trimethylpentene

Synonyms: Pentene, 2,4,4-trimethyl.

Diisobutylene

2,4,4-Trimethylpenten

Diisobutylen

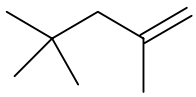
Diisobutene

Molecular weight: 112 g/mol

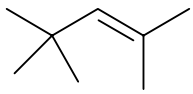
Empirical formula: C_8H_{16}

Structural formula:

2,4,4-Trimethylpent-1-ene



2,4,4-Trimethylpent-2-ene



Purity/impurities, additives

Purity:

2,4,4-Trimethylpentene is a mixture of two isomers

1. 2,4,4-Trimethylpent-1-ene (CAS-No.: 107-39-1) with a typical content of > 71.0 % (range 70 - 80%)

2. 2,4,4-Trimethylpent-2-ene (CAS-No.: 107-40-4) with a typical content of < 22.4 % (range 15 - 25%)

Impurities:

3,4,4-Trimethylpent-1-ene < 1.2 %

5,5-Diemethyl-trans-hex-2-ene < 5.2 %

5,5-Dimethyl-cis-hex-2-ene < 0.8 %

2,3,3-Trimethylpent-1-ene < 0.7 %

3,4,4-Trimethylpent-2-ene (CAS-No.:598-96-9) < 2.4 %

2,3,4-Trimethylpent-2-ene < 1.9 %

2,3,4-Trimethylpent-1-ene < 1.1 %

2,2-Dimethyl-trans-hex-3-ene < 1.6 %

3-Methyl-2-isopropylbut-1-ene < 0.8 %

other C-8-Alkyl-isomers < 4.5 %

Table 1: Physico-chemical properties

Physical form	colourless liquid at 20 °C, 10.13 kPa	RÖMPP Chemie Lexikon Online (2007)
Melting point	< -50 °C (freezing point)	Shell Research (1997)
Boiling point	101.4 – 103.6 °C at 1 013 hPa (method according to Siwoloboff)	Shell Research (1997)
Relative Density	0.7166 at 20 °C (hydrometer method)	Shell Research (1997)
Vapour pressure	57.90 hPa at 25 °C (static method)	Shell Research (1996)
Henry´s law constant	2.127 * 10^5 Pa * m^3/mol	Lyman et al. (1982)

Surface tension	69.9 mN/m at 20 °C c: ca. 1.6 mg/l (ring method)	Shell Research (1997)
Partition coefficient (logPow)	5.0 at 25 °C (HPLC-method)	Shell Research(1997)
Water solubility	1.8 mg/l at 20 °C (flask method)	Shell Research (1997)
Flash point	-7 °C	CHEMSAFE
Auto flammability	380 °C	CHEMSAFE
Flammability	highly flammable	CHEMSAFE
Explosive properties	not explosive	due to structural reasons
Oxidizing properties	not applicable for liquids	

The physico-chemical properties have all been determined experimentally with the isomeric mixture of 2,4,4-Trimethylpent-1-ene (75 %) and 2,4,4-Trimethylpent-2-ene (25 %).

Vapour Pressure

Nichimen Europe plc and EC Erdölchemie have given vapour pressures of 18 kPa at 50 °C and 10 kPa at 38 °C in their safety data sheets but no further information is stated, e.g. test method, test procedure.

Moreover the vapour pressure of the 2,4,4-Trimethylpentene mixture was determined by Shell using the static method. The investigated temperature range was from 25 °C to 51 °C with the following results:

5.79 kPa at 25 °C
7.59 kPa at 31 °C
9.74 kPa at 36 °C
11.94 kPa at 41 °C
17.84 kPa at 51 °C

The Clausius-Clapeyron equation was not applied to these values for calculating the vapour pressure at 25 °C because an experimentally determined value at this temperature is available.

Surface Tension

The surface tension of an aqueous solution of the concentration of 1.6 mg/l, this is approximately 90 % of the saturation concentration in water, was determined experimentally to be 69.9 mN/m at 20 °C.

Water Solubility

In their safety data sheets Nichimen Europe plc and EC Erdölchemie have given < 100 mg/l at 20 °C as the value of the water solubility but no further information.

Furthermore Shell determined experimentally the water solubility at 20 °C by applying the flask method over a time range of 24 h to 96 h resulting in a value of 1.8 mg/l. This value was used for the risk assessment.

Partition Coefficient

Nichimen Europe plc and EC Erdölchemie have given the value of 4.2 for the log Pow in their safety data sheets. There are two other values for log Kow calculated with the KOWWIN program (Webb, 1998). These values are 4.1 and 4.0 for pentene-1 and pentene-2-isomers, respectively. The study has to be assessed as not valid.

Shell calculated the log Pow value to be 4.6 at 25 °C by applying the program "KOWWIN" version 1.35a of the Syracuse Research Corporation.

In addition a further test was conducted at ambient temperature using the HPLC-Method resulting in a value of 5.0 for the log Pow. This value was used for further calculation.

Classification

- Classification according to Annex I

2,4,4-trimetylpentene (CAS number 25167-70-8) is not included in Annex I. The substance

2,4,4-Trimethylpent-1-ene; CAS-No. 107-39-1 is classified in Annex I as follows:

F	R 11	Highly flammable
N	R 51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

- **Proposed classification related to Environment**

On the basis of the available ecotoxicological data 2,4,4-trimethylpentene (CAS-No. 25167-70-8) needs to be classified and labelled related to Environment as:

N	R 50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

This proposal was agreed by the Technical Committee for New and Existing Substances in December 2007.

- Proposed classification **related to Human Health**

On the basis of the Annex I entry of the substance 2,4,4-trimethylpent-1-ene (CAS-No. 107-39-1) the substance 2,4,4-trimethylpentene (CAS-No. 25167-70-8) should be classified and labelled accordingly related to Human Health:

F	R 11	Highly flammable

Since this Annex I entry does not yet include all categories of danger and qualifying risk phrases that have to be assigned according to the data presented, with reference to Human Health, it is proposed to amend this entry.

The substance 2,4,4-trimethylpentene should be classified as irritant and as harmful and labelled additionally with:

Xi	R 38	Irritating to skin
Xn	R 65	Harmful: May cause lung damage if swallowed

The assessment of the aspiration hazard posed by C6-C14 olefins is not based on EU classification criteria but on human experience summarised within a list of data compiled by the Shell Oil Company resulting in the statement: "Aspiration is a significant hazard with C6-C14 olefins". Similar considerations are mentioned in the Martindale Extra Pharmacopoeia

published in 1982, stating on page 1452 for light petroleum: "Adverse Effects and Treatment. As for Kerosene, light petroleum and petrol, being more volatile than kerosene, are more likely to the inhaled and to cause aspiration pneumonitis. The toxicity of petrol varies with its composition". Hence, on the basis of the experience gained by producers of C8 olefins, we suggest that classification with R65 is warranted.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

2.1.1 Production processes

2,4,4-trimethylpentene (diisobutylene) is produced in two production processes. Both processes are continuous and in both processes iso-butenes are used as feedstock. The complete process takes place in a closed system.

In the first process the original feedstock consists of a stream of butane and butenes coming from a butadiene extraction unit. Absorption of isobutenes from this stream is achieved by means of sulphuric acid. No catalyst system is used. The pressure is maintained at about 7 bar at temperatures between 20 and 35 °C. The oligomerization phase occurs at lower pressure and at temperatures of 100 and 130 °C followed by a decantation phase where acid is separated from crude 2,4,4-trimethylpentene, which, once neutralized, is distilled in two steps in order to get the finished product (Deutsche Shell Chemie, 2002).

In the second process the original feedstock consists of a petrochemical stream of isobutene. 2,4,4-trimethylpentene is produced in a liquid phase reaction by catalytic oligomerization using a strong acidic ion exchanger. The reaction mixture is fractionated by distillation to yield the final product, 2,4,4-trimethylpentene (EC Erdölchemie, 2002).

There are two producers in the European Union (Prod.Site A and Prod.Site B). Depending on the feedstock and the process parameters, compositions of different grades are produced (see chapter 0). The two main components are 2,4,4-trimethylpentene-1 and 2,4,4-trimethylpentene-2 .

2.1.2 Production capacity

In the IUCLID data sheet the production volume of both companies is listed within the range of 10,000 to 50,000 t/a (1992 - 1996). According to the latest information, given by both companies, the annual production volume in 2002 was in the range of 40,000 to 50,000 tonnes. An amount of 4,000 to 5,000 t/a was exported from the EU in the same year. Data about an import into the EU are not available. The production volume used in the risk assessment report is based on the production volume reduced by the export volume. Detailed figures are given in the confidential annexes.

It has to be mentioned, that at Prod.Site B a certain proportion of the produced volume is directly processed on-site (approx. 24%).

2.2 USES

According to the information given by the producers, 2,4,4-trimethylpentene is processed at 25 sites in the EU (15[4]) in amounts between 25 t/a and 9,000 t/a. The total amount processed in the EU is within the range of 40,000 to 50,000 t/a. 2,4,4-trimethylpentene is mainly used as chemical intermediate (> 99%) for the production of 3,5,5-trimethylhexanal (Isononal), 3,5,5-trimethylhexanoic acid, 2,2,4-trimethylpentan and 3,5,5-trimethylhexanol (Römpp Chemie Lexikon, 2006).

The following minor uses are also reported, but are not considered in the risk assessment due to the proportion of < 1% of the total production volume.

According to IUCLID the substance is also used as solvent for paints, lacquers and varnishes.

The SPIN-database (Substances in Preparations in Nordic Countries) provides data about the use of 2,4,4-trimethylpentene in products for Denmark, Sweden and Norway in 2004. In Denmark it has been used for the manufacturing of fabricated metal products, except machinery and equipment, in the amount of 1.1 t. The concentration of 2,4,4-trimethylpentene in these degreasing agents is between 0.20 and 0.25 % w/w. The confidential entries for Sweden and Norway have not been considered due to the negligible tonnage. In addition, the substance is found as a rest monomer in polymers together with phenols and α-methylsterene due to its use as a monomer for polymerisations. Environment Canada informed that 2,4,4-trimethylpentene is also used as fuel additive and antioxidant (personal communication, 1996). Table 2.1 shows the use categories and their percentage of the total use.

Table 2.1 Use categories for the uses of 2,4,4-trimethylpentene

Use	Industry category	Use category	Percentage of total use
Chemical intermediate	003	33	Nearly 100 %
Metal extraction, refining and processing industry	008	49	negligible
Fuel Industry	009	28	negligible
Paints, lacquers and varnishes industry	014	48	negligible
Total			100%

[4] Work on this risk assessment began before enlargement of the EU in 2004. All tonnage data, and references to the 'EU' in this risk assessment report, therefore refer to the former EU of 15 Member States.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

During production and use of 2,4,4-trimethylpentene as intermediate (processing) direct releases into the environment might occur to waste water and air. Due to the physicochemical properties of the substance, the main target compartment is the atmosphere (> 99%). In addition, minor indirect releases are expected to surface water via the sewage treatment plants of the processing sites, to sediment and to soil. The local PECs were calculated according to the Emission Scenario Document (ESD) for intermediates (IC 3) assuming that the sites are emitting to an industrial sewage treatment plant.

3.1.2 Environmental releases

3.1.2.1 Release from production

Direct releases from production only occur to waste water and air. Besides the annual production volume and information about the sewage treatment plant capacity no site-specific information about the annual releases of 2,4,4-trimethylpentene are available. Therefore the releases were calculated using the default values of the TGD. Like mentioned above, at Prod.Site B a certain amount (approx. 24 %) of the produced substance is directly processed. Since the releases of the two steps are emitted at the same site it is not appropriate to consider them separately. Therefore the releases of production and processing are added up for the further assessment. The releases are calculated based on the TGD (IC 3, Table A 1.2, f = 0.003). Detailed figures are given in the confidential annexes.

3.1.2.2 Release from industrial/professional use

2,4,4-trimethylpentene is used as intermediate by 25 sites. Since site-specific information was received only from three sites, releases from the other sites are calculated according to the default values of the TGD IC 3 (for releases to waste water: table A 3.3, f = 0.007; for releases to air: table A 3.3, MC = 1c, f = 0.001).

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

Only a small dataset is available to characterize the behaviour of 2,4,4-trimethylpentene in the environment. The tested substance is a mixture of 70 % 2,4,4-trimethylpentene-1 (CAS no: 000107-39-1) and 20 % 2,4,4-trimethylpentene-2 (CAS no: 000107-40-4) and 8 % C8-ole-

fins. Sometimes C8-olefins or other olefins are used as test substance instead of 2,4,4-trimethylpentene.

In the IUCLID data set sometimes 2,2,4-trimethylpentene is cited as test substance instead of 2,4,4-trimethylpentene.

3.1.3.1.1 Atmospheric degradation

At present no experimental data regarding atmospheric degradation are available. For the estimation of the atmospheric degradation the generally accepted QSAR model packages PropertEst and EPI Suite, both including AOPWIN version 1.9, were used. The result of the half life of 7.24 h for indirect photo transformation by OH radicals (500 000 OH radicals/cm³) is the same for both models. Only degradation in the gaseous phase is considered. However, due to the chemical structure of the substance the predictability of the model can be considered as high (Atkinson, 1990).
In addition, a half life of 22.92 h was calculated for photo transformation by ozone molecules ($7 * 10^{11}$ mol/cm³).
In the risk assessment the half life of 7.24 h is used. Consequently, possibly emitted 2,4,4-trimethylpentene will be degraded rapidly in the air.

Furthermore a less exact half life of < 1 day (500 000 OH radicals/cm³) for the indirect photo transformation by OH radicals in the troposphere is listed in the IUCILD datasheet (2000).

3.1.3.1.2 Aquatic degradation

Biodegradation

Table 3.1 Biodegradation data

Test	Observed effects	Author	Reliability according to Klimisch
MITI-List, Closed-Bottle-Test	0 % biodegradation (BOD)	Japan Chemical Industry Ecology-Toxicology & Information Center; 1992	1
OECD 301 D	biodegradation in the range from -10 % to 4 % (ThOD)	Battersby, N.S.; 1996	3
BOD5/COD	BOD5/COD = 0.12	IUCLID; 1998	4

Two studies about the biodegradation behaviour of 2,4,4-trimethylpentene are available.

One is given at the MITI-list of Japan from 1992 (Japan Chemical Industry Ecology-Toxicology & Information Center, 1992). A Closed-Bottle-Test (legal regulation not mentioned) with diisobutylene (CAS no: 107-39-1), not a MITI-test, was performed. Diisobutylene is a synonym for a mixture of 2,4,4-trimethylpentene-1/2. In this special case 2,4,4-trimethylpentene-1 seems to be the only test substance. Concentrations of 1.1 mg/l and 7.2 mg/l diisobutylene respectively together with 2 mg/l activated sludge from an industrial sewage treatment plant (STP) were used. There is no information about the reference

substance, an inoculum control or the number of replicates in the test. Test result was 0 % biodegradation (BOD) of 2,4,4-trimethylpentene-1.

Another available Closed-Bottle-Test (OECD 301 D) was conducted by Battersby (1996). A mixture of 2,4,4- trimethylpentene-1(~75%) and 2,4,4- trimethylpentene-2 (~25%) was used as test substance together with activated sludge from a domestic STP. The test was performed under aerobic conditions. The used concentration was 3 mg/l. As the test substance has a low solubility in water and a high volatility, it was dosed into test flasks by direct injection. After 28 d a biodegradation in the range from -10 % to 4 % (ThOD) was measured. Therefore 2,4,4-trimethylpentene is assessed as not readily biodegradable after 28 days. The rate constant k is therefore 0 h^{-1}. The toxicity control (sodium benzoate as reference substance + 3 mg/l 2,4,4-trimethylpentene + activated sludge) shows a biodegradation < 25 % (based on ThOD) after 14 d. Furthermore an inhibition to microbial activity at the concentration of 3 mg/l was determined. Due to that the test has to be assessed as not reliable.

In the IUCLID data set the BOD_5 is given as 0.19 mg O_2/l, the COD is cited to be 1.59 mg/g substance. From the relationship between BOD_5 and COD an estimation of biodegradation is possible, but is not of the same value as a biodegradation study. Since the original reference is not available, it is not possible to assess the calculation of the BOD_5/COD ratio and the result of 0.12 listed in the IUCLID data set. Therefore the data are assessed as not assignable.

Based on the available information a sound assessment of biodegradation is not possible, so as a worst-case 2,4,4-trimethylpentene has to be assessed as not biodegradable.

Hydrolysis:

Based on the molecular structure hydrolysis of 2,4,4-trimethylpentene is not expected at environmental conditions. Tests concerning hydrolysis are not available.

3.1.3.1.3 Degradation in soil

No data about the degradation in soil are available.

3.1.3.2 Distribution

According to the TGD the vapour pressure and the water solubility have to be corrected to the environmental temperature of 12 °C. Based on the validated vapour pressures of 5790 Pa at 25 °C and 4550 Pa at 20 °C a specific enthalpy of vaporisation of 34.99 kJ/mol was estimated in accordance to Clausius-Clapeyron. Using this estimated figure a vapour pressure of 3040 Pa at 12 °C and a water solubility of 1.604 mg/l at 12 °C was estimated. The results listed in Table 3.2 show the theoretical distribution between the environmental compartments based on the fugacity model “Level I” developed by D. Mackay (D. Mackay, 2001).

Table 3.2 Distribution of 2,4,4-trimethylpentene

Compartment	Distribution [%]
Air	99.99
Water	0.0013
Soil	$1.41 * 10^{-4}$
Sediment	$1.43 * 10^{-4}$
Suspended matter	$5.48 * 10^{-7}$
Fish	$4.61 * 10^{-6}$
Aerosol	$7.33 * 10^{-19}$

Based on these data, the target compartment for the steady-state distribution is the atmosphere.

3.1.3.2.1 Adsorption

The adsorption coefficient log Koc of 2,4,4-trimethylpentene was determined as 2.75 by the HPLC screening test method (draft of test guideline C.19 of Annex V to Directive 67/548/EEC). Furthermore a log Koc value of 2.44 for both isomers of 2,4,4-trimethylpentene was calculated using the PCKOCWIN program (version 1.62) (Webb, 1998).

Based on the validated log Kow value of 5.0 a log Koc of 4.15 was estimated using the equation for hydrophobics from the TGD. Based on the measured log Koc the mobility of 2,4,4-trimethylpentene in soil can be classified as low (Blume, Ahlsdorf, 1993).
Since the measured log Koc value of 2.75 is considered as most reliable it was chosen for the calculation in the risk assessment. Based on that figure and the default organic carbon contents as proposed in table 3 of chapter 3 of the TGD the partition coefficients for each compartment were calculated (Table 3.3).

Table 3.3 Partition coefficients

Parameters	Organic carbon content [%]	Partition coefficients [l/kg]
Kp (soil)	2	11.25
Kp (sediment)	5	28.12
Kp (suspended matter)	10	56.23
Kp (raw sewage)	30	168.70

Kp (activated sludge)	37	208.07

3.1.3.2.2 Volatilisation

The Henry's law constant of 2.127 * 10^5 Pa * m³/mol, calculated from the environmental physical and chemical properties as presented in chapter 3.1.3.2, indicates that the volatility from water is very high (Lyman et al., 1982).

3.1.3.2.3 Distribution in wastewater treatment plants

Using the simulation model SimpleTreat 3.0 (debugged version February 1997) with the above estimated partition coefficients for raw sewage and activated sludge, the Henry's law constant as well as a biodegradation rate of 0 hr^{-1}, the elimination was estimated. In the following table the results are listed.

Table 3.4 Elimination of 2,4,4-trimethylpentene in waste water treatment plant

Fraction directed to air	90.5 %
Fraction directed to water	4.8 %
Fraction directed to sludge	4.7 %
Fraction degraded	0.0 %
Total removal from waste water	95.2 %

The main portion of 2,4,4-trimethylpentene is directed into the atmosphere. The fractions directed to sludge and water are each < 5 %.

3.1.3.3 Bioaccumulation

3.1.3.3.1 Partition coefficient, log K_{ow}

Measured and calculated values are available for the log Kow. In a study from Davies and Webb (1996) the physicochemical properties of 2,4,4-trimethylpentene have been determined in accordance with the test methods described in the Official Journal of the European Communities, L383A, Vol. 35, 1992 (Annex V of RL 92/69/EWG). The "shake-flask" method (A 8) was used for the determination of the log Kow resulting in a value of 4.6. In the same study a log Kow of 4.4 was calculated using the KOWWIN program.

In 1997 Webb and Eadsforth determined a log Kow of 4.9 and 5.0 respectively using a reverse-phase HPLC. A third study conducted by Webb in 1998 gives another log Kow value calculated with KOWWIN. The results are 4.0 and 4.1, but the values obtained using the HPLC method are considered more reliable.

As all values are > 3, there is an indication of a bioaccumulation potential. Due to the higher reliability of the HPLC-measured value compared to the others, the log Kow value used in the further assessment is 5.0.

3.1.3.3.2 Bioaccumulation in fish

Only one study on bioaccumulation in aquatic organisms is available. In the MITI-list of Japan from 1992 the bioaccumulation of 2,4,4-trimethylpentene-1 in *Cyprinus carpio*, was examined. According to the authors the used guideline „Bioaccumulation test of chemical substance in fish and shellfish" corresponds to the OECD guideline 305C. In the publication only a summary is given (Reliability Code = 2). The reported details are log Kow: 4.55, lipid content of the test organisms: 4.7 % and the used test concentrations are 2.5 and 25 µg/l. A BCF of 350 – 868 was determined.

It should also be kept in mind that for a sound assessment of the bioaccumulation potential additional information like Ct50 (clearance time), metabolism, incomplete elimination and organ-specific accumulation are necessary.

Besides these limitations the BCF of 868 is used for a preliminary assessment.

3.1.4 Aquatic compartment (incl. sediment)

3.1.4.1 Calculation of predicted environmental concentrations (PEClocal)

3.1.4.1.1 Calculation of PEClocal for production

There are only two companies producing 2,4,4-trimethylpentene. As previously mentioned, at Prod.Site B a certain amount of the produced 2,4,4-trimethylpentene is directly processed. Therefore the PEC-values for Prod.Site B are derived from the emissions from the production process and the emissions from the use as intermediate together. For descriptive reasons Prod.Site B is listed under “production”. The PEC_{local} was calculated according to the ESD IC 3. Based on these PEC_{local}-values for surface water the PEC_{local} for sediment were calculated as follows:

$$PEClocal_{sed} = \frac{Ksusp\text{-}water}{RHOsusp} \cdot PEClocal_{water} \cdot 1000$$

Tab 3.5 Clocal $_{effluent}$, PEC_{local} (water)and PEC_{local} (sediment) for production

Company	Clocal effluent [µg • l^{-1}]	PEClocal (water) [µg • l^{-1}]	PEClocal (sediment) [µg • kg^{-1}]
Prod.Site A	1010	25.2	328
Prod.Site B	1691	42.3	550

For Prod.Site B specific information about the duration of emission for production and processing was used.

3.1.4.1.2 Calculation of PEClocal for industrial use

For three companies **site specific data** about the effluent flow and the receiving water flow are available (**Proc.Site A, B and C**) and were considered in the PEC_{local} calculation. For the other 22 processing companies PEC_{locals} are calculated according to the ESD IC 3. Based on these PEC_{locals} for surface water the PEC_{locals} for the sediment were calculated as above.

Table 3.6 Clocal $_{effluent}$, PEC local (water) and PEC local (sediment) for use as intermediate

Company	Clocal $_{effluent}$ [µg • l^{-1}]	PEC_{local} (water) [µg • l^{-1}]	PEC_{local} (sediment) [µg • kg^{-1}]
Proc.Site A	0.4	0.003	0.03
Proc.Site B	14.84	0.02	0.26
Proc.Site C	3380	560	7.280
Proc.Site D	134.4	3.4	43.7
Proc.Site E	551.0	14	179
Proc.Site F	2880.0	72	937
Proc.Site G	134.4	3.4	43.7
Proc.Site H	134.4	3.4	43.7
Proc.Site I	208.3	5.2	67.8
Proc.Site J	134.4	3.4	43.7
Proc.Site K	84.0	2.1	27.3
Proc.Site L	84.0	2.1	27.3
Proc.Site M	134.4	3.4	43.7
Proc.Site N	133.3	3.3	43.3
Proc.Site O	84.0	2.1	27.3
Proc.Site P	133.3	3.3	43.3
Proc.Site Q	84.0	2.1	27.3
Proc.Site R	526.4	13	171
Proc.Site S	134.4	3.4	43.7
Proc.Site T	84.0	2.1	27.3
Proc.Site U	134.4	3.4	43.7
Proc.Site V	134.4	3.4	43.7
Proc.Site W	399.8	10	130
Proc.Site X	134.4	3.4	43.7
Proc.Site Y	134.4	3.4	43.7
Others	84.0	2.1	26.8

3.1.4.2 Measured levels

Monitoring data for waste water and surface water are not available.

3.1.5 Terrestrial compartment

3.1.5.1 Calculation of predicted environmental concentrations (PEClocal)

Due to the high volatility release to soil may occur by deposition from atmosphere. The main part of 2,4,4-trimethylpenten entering the waste water treatment plant is distributed into air (90,5%) and just a minor part of 4.7 % adsorbs to sludge. Spreading of contaminated sewage sludge is not considered as all sites are assumed to be connected to an industrial wwtp. Therefore it is assumed that sludge is incinerated.
For the calculation of the local PEC only deposition from air was considered. Site-specific information was not available for the calculation.

3.1.5.1.1 Calculation of PEClocal for production

In Table 3.7 the deposition rate and the local PECs for the production sites are compiled.

Table 3.7 PEC local based on measured Koc

	DEP_{total_ann}	PEC local for					
	($mg/m^2 \cdot d^1$)	soil (mg/kg)	agric. Soil (mg/kg)	grassland (mg/kg)	soil porewater (mg/l)	agric. soil porewater (mg/l)	grassland porewater (mg/l)
Prod.Site A	0.219	$1.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$	$1.1 \cdot 10^{-4}$	$5.1 \cdot 10^{-6}$	$5.1 \cdot 10^{-6}$	$5.1 \cdot 10^{-6}$
Prod.Site B	0.329	0.0002	0.0002	0.0002	$7.7 \cdot 10^{-6}$	$7.7 \cdot 10^{-6}$	$7.7 \cdot 10^{-6}$

3.1.5.1.2 Calculation of PEClocal for industrial/professional use

Table 3.8 lists the deposition rate and the PEC_{local} values for the terrestrial compartment for the industrial use as intermediate.

Table 3.8 PEC local based on measured Koc

	DEP_{total_ann}	PEC local for					
	($mg/m^2 \cdot d^1$)	soil (mg/kg)	agric. Soil (mg/kg)	grassland (mg/kg)	soil porewater (mg/l)	agric. soil porewater (mg/l)	grassland porewater (mg/l)
Proc.Site A	0.0009	$4.4 \cdot 10^{-7}$	$4.4 \cdot 10^{-7}$	$4.4 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.1 \cdot 10^{-8}$	$2.1 \cdot 10^{-8}$
Proc.Site B	0.011	$5.3 \cdot 10^{-6}$	$5.3 \cdot 10^{-6}$	$5.3 \cdot 10^{-6}$	$2.6 \cdot 10^{-7}$	$2.6 \cdot 10^{-7}$	$2.6 \cdot 10^{-7}$
Proc.Site C	0.054	$2.6 \cdot 10^{-5}$	$2.6 \cdot 10^{-5}$	$2.6 \cdot 10^{-5}$	$1.3 \cdot 10^{-6}$	$1.3 \cdot 10^{-6}$	$1.3 \cdot 10^{-6}$
Proc.Site D	0.0012	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$
Proc.Site E	0.03	$1.4 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$6.9 \cdot 10^{-7}$	$6.9 \cdot 10^{-7}$	$6.9 \cdot 10^{-7}$
Proc.Site F	0.036	$1.7 \cdot 10^{-5}$	$1.7 \cdot 10^{-5}$	$1.7 \cdot 10^{-5}$	$8.4 \cdot 10^{-7}$	$8.4 \cdot 10^{-7}$	$8.4 \cdot 10^{-7}$
Proc.Site G	0.006	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$
Proc.Site H	0.0012	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$
Proc.Site I	0.011	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$
Proc.Site J	0.0048	$2.3 \cdot 10^{-6}$	$2.3 \cdot 10^{-6}$	$2.3 \cdot 10^{-6}$	$1.1 \cdot 10^{-7}$	$1.1 \cdot 10^{-7}$	$1.1 \cdot 10^{-7}$
Proc.Site K	0.0003	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$
Proc.Site L	0.0007	$3.5 \cdot 10^{-7}$	$3.5 \cdot 10^{-7}$	$3.5 \cdot 10^{-7}$	$1.7 \cdot 10^{-8}$	$1.7 \cdot 10^{-8}$	$1.7 \cdot 10^{-8}$
Proc.Site M	0.0012	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$
Proc.Site N	0.0015	$7.2 \cdot 10^{-7}$	$7.2 \cdot 10^{-7}$	$7.2 \cdot 10^{-7}$	$3.5 \cdot 10^{-8}$	$3.5 \cdot 10^{-8}$	$3.5 \cdot 10^{-8}$
Proc.Site O	0.0003	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$
Proc.Site P	0.002	$7.2 \cdot 10^{-7}$	$7.2 \cdot 10^{-7}$	$7.2 \cdot 10^{-7}$	$3.5 \cdot 10^{-8}$	$3.5 \cdot 10^{-8}$	$3.5 \cdot 10^{-8}$
Proc.Site Q	0.0003	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$1.5 \cdot 10^{-7}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$	$7.0 \cdot 10^{-9}$
Proc.Site R	0.028	$1.3 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$	$6.5 \cdot 10^{-7}$	$6.5 \cdot 10^{-7}$	$6.5 \cdot 10^{-7}$
Proc.Site S	0.0012	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$5.8 \cdot 10^{-7}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$	$2.8 \cdot 10^{-8}$
Proc.Site T	0.0024	$1.2 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	$5.6 \cdot 10^{-8}$	$5.6 \cdot 10^{-8}$	$5.6 \cdot 10^{-8}$
Proc.Site U	0.006	$2.9 \cdot 10^{-6}$	$2.9 \cdot 10^{-6}$	$2.9 \cdot 10^{-6}$	$1.4 \cdot 10^{-7}$	$1.4 \cdot 10^{-7}$	$1.4 \cdot 10^{-7}$
Proc.Site V	0.0006	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$
Proc.Site W	0.002	$1.1 \cdot 10^{-5}$	$1.1 \cdot 10^{-5}$	$1.1 \cdot 10^{-5}$	$5.1 \cdot 10^{-7}$	$5.1 \cdot 10^{-7}$	$5.1 \cdot 10^{-7}$
Proc.Site X	0.006	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$2.9 \cdot 10^{-7}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$
Proc.Site Y	0.005	$2.3 \cdot 10^{-6}$	$2.3 \cdot 10^{-6}$	$2.3 \cdot 10^{-6}$	$1.1 \cdot 10^{-7}$	$1.1 \cdot 10^{-7}$	$1.1 \cdot 10^{-7}$
Others	0.0005	$2.2 \cdot 10^{-7}$	$2.2 \cdot 10^{-7}$	$2.2 \cdot 10^{-7}$	$1.1 \cdot 10^{-8}$	$1.1 \cdot 10^{-8}$	$1.1 \cdot 10^{-8}$

3.1.6 Atmosphere

3.1.6.1 Calculation of predicted environmental concentrations (PEClocal)

The calculations of PEC_{local} for the atmosphere were carried out according to the A- and B-tables of the TGD with the model OPS. Specific data for releases into the atmosphere are not available.

3.1.6.1.1 Calculation of PEClocal for production

Tabel 3.9 shows the PEC_{local} for production.

Table 3.9 **PEC_{local} and deposition rates for production**

Company	$PEClocal_{air\_ann}$ [mg • m^{-3}]
Prod.Site A	0.22
Prod.Site B	0.16

3.1.6.1.2 Calculation of PEClocal for industrial/professional use

Tabel 3.10 shows the PEC_{local} for the use as intermediate.

Table 3.10 **PEC_{local} and deposition rates for industrial use**

Company	$PEClocal_{air\_ann}$ [mg • m^{-3}]
Proc.Site A	$7.282 \cdot 10^{-4}$
Proc.Site B	$9.171 \cdot 10^{-3}$
Proc.Site C	0.043
Proc.Site D	$9.695 \cdot 10^{-4}$
Proc.Site E	0.024
Proc.Site F	0.029
Proc.Site G	$4.87 \cdot 10^{-4}$
Proc.Site H	$9.695 \cdot 10^{-4}$
Proc.Site I	$8.979 \cdot 10^{-3}$
Proc.Site J	$3.864 \cdot 10^{-3}$
Proc.Site K	$2.457 \cdot 10^{-4}$
Proc.Site L	$5.835 \cdot 10^{-4}$
Proc.Site M	$9.695 \cdot 10^{-4}$
Proc.Site N	$1.211 \cdot 10^{-3}$
Proc.Site O	$2.457 \cdot 10^{-4}$
Proc.Site P	$1.211 \cdot 10^{-3}$
Proc.Site Q	$2.457 \cdot 10^{-4}$
Proc.Site R	0.023
Proc.Site S	$9.695 \cdot 10^{-4}$
Proc.Site T	$1.934 \cdot 10^{-3}$
Proc.Site U	$4.829 \cdot 10^{-3}$
Proc.Site V	$4.87 \cdot 10^{-4}$
Proc.Site W	0.017
Proc.Site X	$4.829 \cdot 10^{-3}$
Proc.Site Y	$3.768 \cdot 10^{-3}$
Others	$3.663 \cdot 10^{-4}$

3.1.7 Non compartment specific exposure relevant to the food chain

Due to log Kow and the bioconcentration factor of 868 for fish a bioaccumulation via the aquatic food chain can not be excluded. For an exposure scenario it is assumed that 50 % of the diet comes from a local area and 50 % from a regional area. According to the TGD the predicted environmental concentration ($PEC_{oral,predator}$) is calculated from the annual PECwater (50 % PEC_{local} + 50 % $PEC_{regional}$), the measured BCF and the biomagnification factor (BMF).

$PEC_{oral,predator} = PEC_{water}$ [mg/l] · BCF_{fish} [$l/kg_{wet\ fish}$] · BMF
$PEC_{oral,predator} = PEC_{water} \cdot 868 \cdot 1$

The following table shows the modified PEC_{water} and the derived $PEC_{oral,predator}$. Regarding the BMF the default value of 1 was chosen due to the BCF of < 2000.

Table 3.11 PEC_{oral} for industrial use and processing

Company	modified PEC (water) [µg/l]	PEC oral, predator [$mg/kg_{wet\ fish}$]
Prod.Site A	10.4	9.0
Prod.Site B	36.1	31.4
Proc.Site A	0.001	0.001
Proc.Site B	0.01	0.009
Proc.Site C	94.3	81.9
Proc.Site D	0.2	0.2
Proc.Site E	5.7	4.9
Proc.Site F	6.9	6.0
Proc.Site G	0.1	0.1
Proc.Site H	0.2	0.2
Proc.Site I	2.1	1.9
Proc.Site J	0.9	0.8
Proc.Site K	0.1	0.1
Proc.Site L	0.1	0.1
Proc.Site M	0.2	0.2
Proc.Site N	0.3	0.3
Proc.Site O	0.1	0.1
Proc.Site P	0.3	0.3
Proc.Site Q	0.1	0.1
Proc.Site R	5.4	4.7
Proc.Site S	0.2	0.2
Proc.Site T	0.5	0.4
Proc.Site U	1.2	1.0
Proc.Site V	0.1	0.1
Proc.Site W	4.1	3.6
Proc.Site X	1.2	1.0
Proc.Site Y	0.9	0.8
Others	0.1	0.1

For the risk assessment it is assumed, that there is no spreading of contaminated sewage sludge. Therefore deposition from air is the only exposure route for the terrestrial compartment. Based on the derived PEC-values (see Tables 3.7/3.8) for the terrestrial compartment (in the range between 10^{-5} and 10^{-7} mg/kg) releases into soil are considered as negligible. Hence, bioaccumulation via the terrestrial food chain is considered as not relevant.

3.1.8 Calculation of PECregional and PECcontinental

To estimate the regional background concentrations, all releases are taken into account. A share of 90 per cent of the total releases was allocated to the continental scale, whilst 10 per cent are allocated to the regional sector. The software program EUSES 2.0 was used to calculate regional concentrations. In the table below the regional and continental PECs are compiled.

Table 3.12 PECregional and PECcontinental based on the measured Koc

continental PECs		**regional PECs**	
$PECcont_{surfacewater}$	$5.36 \cdot 10^{-12}$ mg/l	$PECreg_{surfacewater}$	$3.1 \cdot 10^{-10}$ mg/l
$PECcont_{air}$	3.65 • 10-^{07} mg/m^3	$PECreg_{air}$	$4.47 \cdot 10^{-06}$ mg/m^3
$PECcont_{agrsoil}$	$9.71 \cdot 10^{-11}$ mg/kg	$PECreg_{agrsoil}$	$1.19 \cdot 10^{-09}$ mg/kg
$PECcont_{agrsoilporew}$	$5.38 \cdot 10^{-12}$ mg/l	$PECreg_{agrsoilporew}$	$6.59 \cdot 10^{-11}$ mg/l
$PECcont_{natsoil}$	$9.68 \cdot 10^{-11}$ mg/kg	$PECreg_{natsoil}$	$1.19 \cdot 10^{-09}$ mg/kg

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

Available effect data

In the following table the available effect data for 2,4,4-trimethylpentene are summarized.

Table 3.13 Ecotoxicological effect data.

Species		Observed effect	Source	Reliability
Fish	*Oncorhynchus mykiss*	96 h-LC_{50} = 0.58 mg/l	Eadsforth, Palmer, Sherren, 1996	1
	Leuciscus idus	not assignable / not valid (open test vessels, slow aeration, nom. concentration)	Eadsforth, Palmer, Sherren, 1996	4
	Oryzias latipes	48 h-LC_{50} = 2.15 mg/l	MITI Japan, 1992	4
Invertebrates	*Daphnia magna*	48 h-LC_{50} = 1.2 mg/l	Eadsforth, Palmer, Sherren, 1996	1
Algae	*Selenastrum capricornutum*	72 h-E_bC_{50} = 0.86 mg/l 72 h-E_rC_{50} = 1.67 mg/l	Eadsforth, Palmer, Sherren, 1996	1
Micro-organisms	*Pseudomonas fluorescens*	not assignable / not valid (documentation insufficient for assessment)	Shell Chemicals. 1994	4
	Activated sludge (closed bottle test)	indication of inhibition of microbial activity at 3 mg/l	Battersby, 1996	3
Terrestrial organisms	No test results available			

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Toxicity test results

Concerning the ecotoxic effects of 2,4,4-trimethylpentene on aquatic organisms only a small dataset is available. Due to the volatility and low water solubility of the substance it is important to prevent loss of the test substance by volatilization during testing. In addition to that, test results need to be considered as measured concentrations.

3.2.1.1.1 Fish

In a sealed, semi-static test, Eadsforth et al. (1996) tested the acute toxicity of 2,4,4-trimethylpentene to the rainbow trout *Oncorhynchus mykiss.* A 50:50 mixture of two products containing both 2,4,4-trimethylpentene-1 and 2,4,4-trimethylpentene-2 isomers was used as test substance. The study was performed according to OECD Guideline 203. Fish were exposed for 96 h with daily replacement of the test water (tap water, hardness 135-159 mg/l $CaCO_3$, pH 7.1-7.4, dissolved oxygen 8.6-9.6 mg/l, temperature 14-15°C). Five test concentrations spaced by a constant factor of 2.2 were used. The highest concentration tested was 7.1 mg/l (measured concentration). The following effect values derived from the analytically verified concentrations were determined:

Table 3.14 Acute toxicity in fish (*Oncorhynchus mykiss*)

exposure period [h]	effect cocentration [mg/l]	95%-CI	effect	NOEC [mg/l]
24	24 h-LC_{50} = 1.2	0.81 - 2.3	mortality	-
48	48 h-LC_{50} = 0.65	0.37 - 0.99	mortality	-
72	72 h-LC_{50} = 0.58	0.32 - 0.88	mortality	-
96	96 h-LC_{50} = 0.58	0.32 - 0.88	mortality	96 h-NOEC = 0.18

Two more effect values for fish are available. A study with *Leuciscus idus* from Eadsforth et al. (1996) and a study with *Oryzias latipes* from the MITI-List Japan (1992). Both studies have to be assessed as not assignable. Eadsforth et al used open test vessels with slow aeration resulting in a considerable loss of test substance. Furthermore, only nominal concentrations were reported. The data from the MITI-list seem to be reliable, but no information about the used test guideline has been available.

3.2.1.1.2 Invertebrates

Eadsforth et al.(1996) studied the effects of short-term exposure of *Daphnia magna* to 2,4,4-trimethylpentene according to OECD Guideline 202 "Daphnia sp., Acute Immobilization Test" with a mixture of two 2,4,4-trimethylpentene isomers (2,4,4-trimethylpente-1 and 2,4,4-trimethylpente-2) as test substance. Young daphnids were exposed for 48 h in a static and closed system using reconstituted freshwater with a hardness of 160 mg/l $CaCO_3$, pH 8.1-9.5, dissolved oxygen 8.5-9.2 mg/l and temperature 20-21°C. Five test concentrations spaced by a constant factor of 2.2 were employed; the highest concentration tested was 8.4 mg/l (effective concentration). The derived effect values are based on measured concentrations and are given below.

Table 3.15 Acute toxicity in invertebrates (*Daphnia magna)*

exposure period [h]	effect cocentration [mg/l]	95%-CI	effect	NOEC [mg/l]
24	24 h-LC_{50} = 2.1	1.7 - 2.7	immobilisation	-
48	48 h-LC_{50} = 1.2	0.92 - 1.5	immobilisation	48 h-NOEC = 0.56

3.2.1.1.3 Algae

In a 72-hour static test conducted according to OECD Guideline 201 "Algae, Growth Inhibition Test" the acute toxicity of 2,4,4-trimethylpentene (mixture of two isomers as described above) to the green algae *Selenastrum capricornutum* was examined by Eadsforth et al.(1996). The test was performed in a closed system with 7 test concentrations (pH 6.3-10.0, temperature 23-24°C, constant illumination). The highest concentration tested was 9.0 mg/l (effective concentration). Growth inhibition of the algae was measured with a Coulter Multisizer. Effect data were related to measured concentrations.

The original test data were reanalysed using the UBA ToxTool[5] (by means of probit analysis).

Table 3.16 Toxicity in algae (*Selenastrum capricornutum*)

time [h]	effects on growth rate [mg/l]	effects on biomass [mg/l]
24	24-E_RC_{10} = 0.35 (95%-CI: 0.04 - 0.66)	24-E_BC_{10} = 0.14 (95%-CI: 0.00 - 0.39)
	24-E_RC_{50} = 1.67 (95%-CI: 0.96 - 3.23)	24-E_BC_{50} = 1.05 (95%-CI: 0.37 - 4.38)
48	48-E_RC_{10} = 0.69 (95%-CI: 0.28 - 0.99)	48-E_BC_{10} = 0.36 (95%-CI: n.d.)
	48-E_RC_{50} = 1.56 (95%-CI: 1.11 - 2.23)	48-E_BC_{50} = 1.07 (95%-CI: n.d.)
72	72-E_RC_{10} = 0.75 (95%-CI: 0.36 - 1.05)	72-E_BC_{10} = 0.17 (95%-CI: n.d.)
	72-E_RC_{50} = 1.67 (95%-CI: 1.24 - 2.27)	72-E_BC_{50} = 0.86 (95%-CI: n.d.)

n.d.: not determined due to mathematical reasons

3.2.1.1.4 Microorganisms

A biodegradation study according to the OECD Test Guideline 301D: Closed Bottle Test was conducted by Battersby (1996). As test substance a mixture of the two 2,4,4-trimethylpentene isomers (75% 2,4,4-trimethylpentene-1 and 25% 2,4,4-trimethylpentene-2) and activated sludge from a domestic sewage treatment plant was used as inoculum. The concentration of 2,4,4-trimethylpentene applied directly into the test flasks was 3 mg/l. The toxicity control including sodium benzoate as reference substance, activated sludge and 3 mg/l 2,4,4-trimethylpentene showed a biodegradation of < 25% (based on ThOD) which indicates an inhibition of microbial activity at this level. No dose-response relationship could be derived from the test system. The test has to be assessed as not reliable based on the scoring system of Klimisch at al. (Reliability code = 3).

Another test concerning the toxicity of 2,4,4-trimethylpentene to *Pseudomonas fluorescens* was conducted by the Bayer AG (1978) and is listed in the IUCLID Data Set (2000). The estimated NOEC is > 100 mg/l after 24 h. The documentation of the test is insufficient for an assessment. No information is given about the type of test and the test method (Reliability code = 4).

[5] Developed by ToxRat Solutions GmbH, Germany

3.2.1.2 Predicted toxicity data

Predicted toxicity data are used in order to provide some information about the possible acute mode of action of the substance and in order to give some indications how risk characterization might change if chronic toxicity values would be available.

Based on the chemical structures of 2,4,4-trimethylpent-1-ene and 2,4,4-trimethylpent-2-ene, both the Verhaar/Hermens reactivity rules and the OASIS Acute Toxicity MOA (included in the software OECD (Q)SAR Application Toolbox) which mainly uses the categorisation rules by Verhaar et al. (1992) and Russom et al. (1997) predicts that the two substances only exert the lowest type of acute toxicity exerted by organic chemicals, i.e. non-polar narcosis/baseline toxicity/minimum toxicity.

Non-polar narcosis/baseline toxicity/inert chemicals are considered to represent the lowest form of toxicity exerted by organic chemicals. However, some of these substances may in addition also exert other types of toxicity (e.g. less inert compounds/polar narcosis, reactive substances or substances acting by specific modes of action), which makes them more toxic than predicted only based upon the non-polar narcosis. Since the prediction of acute toxicity of substances only acting via non-polar narcosis in general is considered to be good, the tricky and crucial part becomes to determine whether or not excess toxicity also can be expected. If so, the use of predictions for non-polar narcosis will underestimate the toxicity of the substance.

A comparison between comparable experimental and predicted data (using equations for non-polar narcosis) for 2,4,4-trimethylpent-1-ene could be used to support an assumption that baseline toxicity is correct.

Table 3.17 includes acute aquatic experimental and predicted toxicity data (unfortunately not for all phylas with identical species).

Table 3.17 Measured and predicted toxicity data

Species	Experimental value	Predicted value
Fish	*Oncorhynchus mykiss* LC_{50} (96h) = 0.58 mg/l	*Pimephales promelas* LC_{50} (96h) = 0.26 mg/l (using $logK_{ow}$ = 5.0 in TGD-equation) *Pimephales promelas* LC_{50} (96h) = 0.13 mg/l (using logKow = 5.0 in ECOSAR-equation; chemical class Neutral Organics*)
Invertebrates	*Daphnia magna* LC_{50} (48h) = 1.2 mg/l	*Daphnia magna* LC_{50} (48h) = 0.10 mg/l (using $logK_{ow}$ = 5.0 in TGD-equation) *Daphnia magna* LC_{50} (48h) = 0.17 mg/l (using logKow = 5.0 in ECOSAR-equation; chemical class Neutral Organics*)
Algae	*Selenastrum capricornutum* E_bC_{50} (72 h) = 0.86 mg/l E_rC_{50} (72 h) = 1.67 mg/l	*Selenastrum capricornutum* EC_{50} growth (72-96h) = 0.07 mg/l (using $logK_{ow}$ = 5.0 in TGD-equation)

		Selenastrum capricornutum EC_{50} growth (96 h) = 0.12 mg/l** (using logKow = 5.0 in ECOSAR-equation; chemical class Neutral Organics*)

* The program ECOSAR assign an organic chemical to one or several chemical classes for which individual (Q)SARs exists. In case no suitable chemical class exists, the program assigns the chemical to the class "Neutral Organics", which contains equations for the lowest kind of toxicity which all substances at least exert. Using the software OECD (Q)SAR Application Toolbox the 2,4,4-trimethylpent-1-ene is not possible to categorize according to the US EPA Chemical Categories (among which "Neutral Organics" is one).

** although no specific information about the data used for the calculation of the QSAR model is available, it can be assumed that the QSAR-model is based on the endpoint biomass.

Considering the outcome of the classification rules by Verhaar/Hermens and Russom et al. and a comparison between the experimental and predicted toxicity data (bearing in mind the properties of the compound, i.e. high volatility and low water solubility, but also the closed test systems and measured concentrations used), it appears probable that at short term exposure only baseline toxicity is exerted. This is further supported by the predictions of no binding to proteins or DNA (using the OECD (Q)SAR Application Toolbox).

However no experimental chronic data are available in order to make assumptions about the chronic mode of action of the substance. Actual concepts (e.g. Verhaar, Russom) are based on the detection of acute mode of actions and are thus not of use for the detection of chronic modes of action. There is some evidence e.g. Ahlers et al, 2006, that a non-polar mode of action correlates with a low acute to chronic ratio (ACR) and thus probably with a non-polar chronic mode of action. However the correlation between acute mode of action, ACR and chronic mode of action still needs verification. In addition information about structure-activity relationships for chronic toxicity is still low and thus QSAR-results for chronic toxicity should be used with care.

Keeping these shortcomings in mind, a very rough QSAR estimation of a minimum chronic toxicity for fish and Daphnia (for which TGD equations are available) might be provided assuming – as a minimum - a chronic narcotic mode of action. The outcome of this prediction should – in accordance with the TGD – not be used as definitive values for a PEC/PNEC refinement, but could be used as an indication whether further testing might increase the PNEC and thus decrease the PEC/PNEC ratio.

Table 3.18 includes QSAR results for chronic toxicity of 2,4,4-trimethylpentene assuming a minimum non-polar narcotic toxicity (results are based on two TGD equations and a logKow of 5.0).

Table 3.18 QSAR results for chronic toxicity of 2,4,4-trimethylpentene assuming a non-polar narcotic mode of action (chronic algae toxicity based on experimental data is included for completeness)

Species	QSAR method	Result
Fish, *Pimephales promelas*	TGD, ELS Test 28 – 32 d, using a logKow of 5.0	NOEC = 0.018 mg/L
Daphnia *Daphnia magna*	TGD, Reproduction test 16-d, Endpoints growth, reproduction using a logKow of 5.0	NOEC = 0.009 mg/L
Algae *Selenastrum capricornutum*	Experimental data, Eadsforth, Palmer, Sherren, 1996	EbC_{10} = 0.170 mg/L ErC_{10} = 0.745 mg/L

Although the software ECOSAR is developed using predicted log Kow-values the measured log Kow of 5 was used. Since the measured value is higher than the predicted one the result of the calculation can be considered as a "worst-case" toxicity.

Noticeable, the predicted 16d NOEC for daphnid reproduction of 9 µg/l, assuming application of AF 10 to give a '(Q)SAR-based predicted PNEC' of 0.9 µg/l, is quite consistent with the actual PNEC of 0.58 µg/l as derived from acute tests results applying AF 1000.

Taken together, the (Q)SAR-calculations for chronic toxicity are not considered as sufficiently reliable for use in PNEC derivation.

For the purpose of long-term testing the species with the lowest acute LC50 value should be examined first according to the TGD. This would in this case be the fish. However the (Q)SAR results for chronic toxicity indicate that daphnids are more sensitive. Additionally, animal welfare is a reason to consider a daphnid test rather than a fish test. Thus the suggestion is, to carry out a 21-day *Daphnia* reproduction study, and use the QSAR analysis to support an assessment factor of 10 on the NOEC from that study.

3.2.1.3 Calculation of Predicted No Effect Concentration (PNEC)

3.2.1.3.1 Determination of $PNEC_{aqua}$

Due to the physicochemical properties of 2,4,4-trimethylpentene only effect values based on analytically verified concentrations were used to calculate PNEC-values. Such results are available from three acute tests conducted under standardized conditions. The relevant LC_{50}-/EC_{50}- values range from 0.58 mg/l (fish) to 1.67 mg/l (algae, growth rate). The most sensitive species was *Oncorhynchus mykiss* with a 96 h-LC_{50} of 0.58 mg/l.

Since only short-term tests using species from three trophic levels are available; an assessment factor of 1000 is applied to this value.

$PNEC_{water}$ = 0.58 mg/l / 1000 = 0.58 µg/l

3.2.1.3.2 Determination of $PNEC_{micro\text{-}organisms}$

Since no specific test on the inhibition of micro-organisms is available, the PNEC is based on the effect observed in the toxicity control of the OECD 301D. This test was assessed as invalid. Therefore the derived PNEC is only used for a preliminary estimation. For a proper assessment of microbal inhibition an OECD 209 inhibition test is needed.

The nominal tested concentration of 3 mg/l is equal to an exposure concentration equal to the water solubility limit (i.e. 1.8 mg/l). For the derivation of the $PNEC_{STP}$ an assessment factor of 100 is applied to the water solubility limit, resulting in a preliminary **$PNEC_{micro\text{-}organism}$ = 0.018 mg/l**.

It should be noted, that the assessment factor of 100 normally applies to an EC50 derived in a standard OECD 209 respiration inhibition study. The inhibition of the microbial respiration that was recorded in the OECD 301D can be considered equivalent to an approximate 50% effect level.

3.2.1.3.3 Determination of $PNEC_{Sediment}$

As no experimental results with benthic organisms are available the $PNEC_{sed}$ can be provisionally calculated using the equilibrium partitioning method.

$$PNECsed = \frac{14.9\ m^3/\cdot m^3}{1150\ kg/m^3} \quad 0.00058\ mg/l \quad \cdot \quad 1000$$

$PNEC_{sed,\ calculated}$ = 0.00754 mg/kg (wwt) = 7.54 µg/kg (wwt)

3.2.2 Atmosphere

3.2.2.1 Biotic effects

Experimental data for fate and behaviour of 2,4,4-trimethylpentene in the atmosphere are not available. Therefore, the data base is considered to be not sufficient for the derivation of a $PNEC_{air}$ for 2,4,4-trimethylpentene. Due to the physicochemical properties of the substance the atmosphere is expected to be the target compartment.

3.2.2.2 Tropospheric ozone formation

The formation of tropospheric ozone involves complicated chemical reactions between NOx and VOC (Volatile Organic Compounds) driven by the solar radiation. In order for these reactions to occur in substantial quantities, meteorological conditions must prevail that prevents dispersion of NOx and hydrocarbons. After a night time accumulation NOx reacts with sunlight to produce NO and highly reactive atomic oxygen. The atomic oxygen may react with many compounds in the air, i.e. O_2 to produce O_3 or VOC to produce free radicals. The time scale of ozone production is such that ozone concentrations may build up over several days under suitable weather conditions, and that a substance and its precursors can be transported over considerable distances (European Commission DG XI, 1998).

There is as yet no consensus on the quantitative yield of these reactions, making modelling of these processes difficult. In addition to the VOC speciation and concentrations, VOC/NOx ratio, solar radiation and meteorological conditions vary from city to city within the EU. Since the environmental conditions differ considerably, a certain concentration of VOC may lead to very different ozone concentrations within the EU. For example European Commission DGXI (European Commission DG XI, 1998) used a simplified EMEP model calculations and showed how a change in the VOC concentration may affect the ozone formation to a small extent in some parts of Europe (NOx limited region), while in other parts of Europe a change in the VOC concentration will lead to a considerable change in the ozone

formation (high NOx regions). Thus there is no simple relationship between the VOC and NOx concentrations and the resulting tropospheric ozone creation. The ozone concentrations may at some places of Europe even be higher at the same VOC concentration and at lower NOx concentrations than may be the case at other places. Likewise the time trends of the tropospheric ozone concentration for Europe in general cannot be forecasted by predicting the future concentrations of VOC and NOx.

Nevertheless, the member countries in UNECE have agreed to use a Photochemical Ozone Creation Potential (POCP) factor system where the individual POCP values are generally presented as relative values where the amount from a certain VOC is divided with the amount of ozone produced from an equally large amount of ethene (Derewent at al., 1996). The POCP approach can be seen as a ranking system where the POCP value for ethene has been set to 100.

$$POCP = \frac{ozone\,increment\,with\ the\,hydrocarbon}{ozone\,increment\,with\ ethene} \times 100$$

For 2,4,4-trimethylpentene no POCP value was found. However, for alkenes Derwent et al. (1996) gave a range of 83 - 118 and Altenstedt J. and Pleijel K. (1998) a range of 72 - 196. The ozone formation potential depends on the branching in the carbon skeleton, influencing the reaction pathways (Derewent et al 1996). In Table 3.18 the POCP of a number of alkenes is given.

Table 3.19) POCP for different alkenes

Substance	POCP	Source
3-Methylbut-1-ene	118.4	Derewent at al. (1996)
2-Pentene	95.3	Derewent at al. (1996)
2-Methyl-1-butene	94 – 101	Altenstedt and Pleijel (1998)
2-Methyl-2-butene	72 – 151	Altenstedt and Pleijel (1998)
2-Pentene	116 – 141	Altenstedt and Pleijel (1998)

To evaluate the relative importance of 2,4,4-trimethylpentene for the creation of tropospheric ozone using the POCP factor system the VOC composition within the region of concern has to be known. For a simple evaluation of the relative importance of the emitted 2,4,4-trimethylpentene for the creation of ozone the VOC composition from industrial sources as well as the VOC composition from other sources e.g. traffic emissions need to be considered. For a more in depth evaluation also the solar radiation and the NOx concentrations have to be taken into account. These will of course vary considerably in Europe, between regions and between individual sites within the region as will also the VOC composition which depends on composition of the regional / local industrial sector and the traffic.

In the following an attempt to evaluate the relative contribution to the ozone creation potential has been performed.

3.2.2.2.1 Creation of tropospheric ozone due to 2,4,4-trimethylpentene based on estimated emissions

As described above the creation of tropospheric ozone is depending on the occurrence of VOC, NOx, solar radiation and thus OH-radicals in a complicated relationship. The VOC composition will be highly variable and depend on the industrial sources, traffic emissions and natural sources. The contribution from production and processing of 2,4,4-trimethylpentene will depend on the composition of local and regional industry.

Therefore, average calculations are likely to underestimate the magnitude of the problem within certain regions with high exposure potential.

The NMVOC (Non-Methane Volatile Organic Compounds) and NOx as ozone precursors emitted in EU15 is shown in the table below.

Table 3.20 Emission of NMVOC in EU15 (EEA - European Environment Agency, 2006)

NMVOC in EU15 (Kilotons)										
1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
34741	34106	33490	32412	31443	30336	29325	28291	27338	26800	26419

The proportion of 2,4,4-trimethylpentene on the total NMVOC emissions can be calculated by relating the emissions of 2,4,4-trimethylpentene from production and processing (approx. 1000 t in 2002) to the total emission of NMVOC in 2002. The proportion of 2,4,4-trimethylpentene rises to about 0.004%.

The POCP equivalence factor for the total NMVOC is not known because the composition of individual NMVOC species is not available. According to Derewent at al. (1996) alkenes appear to be more reactive than other NMVOC, indicated by the high POCP in relation to ethene. Therefore 2,4,4-trimethylpentene may contribute slightly more to the ozone creation than indicated by the proportion relative to total NMVOC.

To conclude 2,4,4-trimethylpentene contributes in the order of 0.004 % to the total NMVOC emission in the EU15. Thus the substance in general only contributes to a small extent to the total SMOG problem. Furthermore, the short half-life in air (t1/2 = approx. 7.24 h) presumably limits the effect of 2,4,4-trimethylpentene on ozone formation. However, since no measured data for 2,4,4-trimethylpentene in the atmosphere and no POCP value were available a significant contribution to ozone formation can not be excluded.

3.2.3 Terrestrial compartment

Data on effects to terrestrial organisms are not available. In an indicative risk assessment for the soil compartment the equilibrium partitioning method can be applied:

$$PNECsoil = \frac{27.2\ m^3/\cdot m^3}{1700\ kg/m^3} \cdot 0.00058\ mg/l \cdot 1000$$

$PNEC_{soil,\ calculated}$ = 0.00927 mg/kg (wwt) = 9.27 µg/kg (wwt)

3.2.4 Non compartment specific effects relevant to the food chain

A biomagnification via food chain can not be excluded. Therefore a $PNEC_{oral}$ was derived from a 28-day rat toxicity study. The NOAEL for 2,4,4-trimethylpentene was considered to be 300 mg/kg bw/day. According to the TGD the NOAEL was converted into a $NOEC_{mammal,food\_chr}$ using a conversion factor (CONV). A conversion factor of 10 was considered as appropriate.

$NOEC_{mammal,food\_chr} = NOAEL_{mammal,food\_chr} \cdot CONV_{mammal}$

$NOEC_{mammal,food\_chr} = 300\ mg/kg\ bw/day \cdot 10\ kg\ bw\ day/kg_{food}$

$NOEC_{mammal,food\_chr} = 3000\ mg/kg_{food}$

The PNECoral was then calculated by applying an assessment factor of 300 (TGD, chapter 3.8.3.5, table 23) to the $NOEC_{mammal,food\_chr}$.

$PNEC_{oral} = NOEC_{mammal,food\_chr} / 300$

$PNEC_{oral} = 3000\ mg/kg_{food} / 300$

$PNEC_{oral}$ = 10 mg/kg_{food}

In the following table the derived PNECs are summarized.

Table 3.21 Derived PNECs for 2,4,4-trimethylpentene.

PNEC water	0.58 µg/L
PNEC micro-organisms	0018 mg/L
PNEC sed, calculated	7.54 µg/kg (wwt)
PNEC soil,calculated	9.27 µg/kg (wwt)
PNEC air	data not sufficient
PNEC oral	10 mg/kg_{food}

3.3 RISK CHARACTERISATION

The derived PEC/PNEC ratios are mainly based on default values according to the TGD.

3.3.1 Aquatic compartment

3.3.1.1 Waste water treatment plants

Tabel 3.22 shows the derived $Clocal_{effl.}$/PNEC-ratios for production and processing. The corresponding $Clocal_{effl.}$-values are listed in table 3.5 and 3.6. The $PNEC_{micro\text{-}organisms}$ was determined to **0.018 mg/l**.

Table 3.22 $Clocal_{effl.}$/PNEC-ratios for waste water treatment plants

Company	PEC/PNEC
Prod.Site A	**56.1**
Prod.Site B	**93.9**
Proc.Site A	< 0.1
Proc.Site B	0.8
Proc.Site C	**187.8**
Proc.Site D	**7.5**
Proc.Site E	**30.6**
Proc.Site F	**160.0**
Proc.Site G	**7.5**
Proc.Site H	**7.5**
Proc.Site I	**11.6**
Proc.Site J	**7.5**
Proc.Site K	**4.7**
Proc.Site L	**4.7**
Proc.Site M	**7.5**
Proc.Site N	**7.4**
Proc.Site O	**4.7**
Proc.Site P	**7.4**
Proc.Site Q	**4.7**
Proc.Site R	**29.2**
Proc.Site S	**7.5**
Proc.Site T	**4.7**
Proc.Site U	**7.5**
Proc.Site V	**7.5**
Proc.Site W	**22.2**
Proc.Site X	**7.5**
Proc.Site Y	**7.5**
Others	**4.7**

For Proc.Site A and Proc.Site B a PEC/PNEC-ratio < 1 was calculated. Therefore conclusion ii applies to these sites.

Conclusion (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.

Beside these sites conclusion i has to be drawn for the waste water treatment plants.

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

Due to the insufficient data base further exposure information should be required (for instance emission factor and effluent concentration). Furthermore, an OECD 209 respiration inhibition study is needed to derive a more reliable $PNEC_{micro\text{-}organism}$.

3.3.1.2 Aquatic environment

Table 3.23 shows the PEC/PNEC ratios for the aquatic environment for production and processing. The calculation is based on a **$PNEC_{water}$ of 0.58 µg/L** and PEC_{water} values from the tables in chapter 3.1.4.1 and 3.1.4.2.

Table 3.23 PEC./PNEC-ratios for the aquatic compartment

Company	PEC/PNEC
Prod.Site A	**43.5**
Prod.Site B	**72.9**
Proc.Site A	< 0.1
Proc.Site B	< 0.1
Proc.Site C	**964.9**
Proc.Site D	**5.8**
Proc.Site E	**23.8**
Proc.Site F	**124.1**
Proc.Site G	**5.8**
Proc.Site H	**5.8**
Proc.Site I	**9.0**
Proc.Site J	**5.8**
Proc.Site K	**3.6**
Proc.Site L	**3.6**
Proc.Site M	**5.8**
Proc.Site N	**5.7**
Proc.Site O	**3.6**
Proc.Site P	**5.7**
Proc.Site Q	**3.6**
Proc.Site R	**22.7**
Proc.Site S	**5.8**

Proc.Site T	**3.6**
Proc.Site U	**5.8**
Proc.Site V	**5.8**
Proc.Site W	**17.2**
Proc.Site X	**5.8**
Proc.Site Y	**5.8**
Others	**3.6**

Two PEC/PNEC-ratios < 1 were calculated (Proc.Site A and Proc.Site B). For these sites conclusion ii has to be drawn.

Conclusion (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.

For all other sites a PEC/PNEC ratio > 1 was calculated. The ratios vary from 3.6 (Proc.Site K, L, O, Q) to 965 (Proc.Site C). For Proc.Site C site specific data about the effluent flow and the receiving water flow were available. This site has to refine the exposure data. For all other sites PEC values were derived by using generic scenarios only. Therefore further exposure data are required.

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

Further information is necessary to refine the exposure scenarios for the sites with PEC/PNEC > 1. Taking into account the large PEC/PNEC ratios for some sites it is recommended to perform as well long term toxicity testing with aquatic organisms in order to refine the aquatic PNEC. The aquatic PNEC is based currently on acute toxicity tests and AF 1000. For the purpose of long-term testing the species with the lowest acute LC50 value should be examined first according to the TGD. This would in this case be the fish. However the (Q)SAR results for chronic toxicity indicate that daphnids are more sensitive. Additionally, animal welfare is a reason to consider a daphnid test rather than a fish test. Therefore it is suggested to carry out a 21-day *Daphnia* reproduction study (OECD 211), and use the QSAR analysis to support an assessment factor of 10 on the NOEC from that study.

3.3.1.3 Sediment

The calculation of the $PEC_{sed}/PNEC_{sed}$- ratios for the sediment is based on the **$PNEC_{sediment}$ of 7.54 µg/kg (wwt)** and the $PEC_{sediment}$ values from the tables in chapter 3.1.4.1 and 3.1.4.2. The equilibrium partitioning method was used to calculate PNEC and PEC for sediment from the freshwater data.. Therefore risk characterisation ratios for sediment are identical to the surface water risk characterisation ratios. According to the TGD the $PEC_{sed}/PNEC_{sed}$-ratio should be increased by a factor of 10 for substances with a logKow > 5. 2,4,4-trimethylpentene just meets this threshold (logKow = 5). However, in this case a measured Koc value has been used for the calculations, which is an order of magnitude lower than the prediction using Kow, so we assume that the additional factor is not necessary.

Only for Proc.Site A and Proc.Site B a PEC/PNEC ratio < 1 could be determined. Therefore conclusion ii applies to these sites.

Conclusion (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.

For all other sites the generic scenario leads to PEC/PNEC ratios > 1. Ratios vary from 3.6 (Proc.Site K, L, Q) to 965 (Proc.Site A). Due to the fact that the $PEC_{sediment}$ values are based on PEC_{water} a refinement of these values will have an effect on the PEC/PNEC-ratio for the sediment as well.

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

3.3.2 Atmosphere

The atmosphere is the main target compartment of 2,4,4-trimethylpenten as a result of the high vapour pressure of 5.79 kPa at 25 °C. The Henry's law constant of 2.127 * 10^5 Pa * m^3/mol indicates that, too. Due to the calculated atmospheric half-life of 2,4,4-trimethylpentene (t1/2 = approx. 7.24 h) the substance will the be degraded rapidly.

Since no data are available about ecotoxicological effects of the substance in connection with the atmosphere, it is not possible to undertake a quantitative risk assessment.

It is known that alkenes contribute to tropospheric VOC and contribute to the tropospheric formation of ozone. The photochemical formation of ozone and other compounds depends on emission of all VOCs and other compounds in a complex interaction with other factors.

Changes in VOC emissions lead to changes in ozone formation. The efficiency of VOC emission reductions in reducing ground level ozone concentrations may vary from place to place and depends on the occurrence of NOx, the solar radiation and the prevailing wind conditions. Thus the effects on ozone creation of emissions arising from the production and processing of 2,4,4-trimethylpentene may differ substantially between different regions in the EU.

Based on a rough estimation given in chapter 3.2.2.2, the current risk assessment indicates that emission of 2,4,4-trimethylpentene from use and processing may be in the order of 0.004 % of total NMVOC emissions. Locally and regionally this proportion may vary substantially due to differences between regions in the VOC emission pattern from industrial sectors using 2,4,4-trimethylpentene. Even a simple evaluation of the photochemical ozone creation potential of 2,4,4-trimethylpentene is difficult to perform, since the concentration in the atmosphere and the specific POCP of 2,4,4-trimethylpentene are not available.

Effects of ozone exposure are well documented on plants, animals and humans. Therefore, the EU has emission control legislation in place to reduce the emission of ozone precursors like

NMVOC (for example the National Emission Ceiling Directive, 2001/81/EC, or the Air Quality Framework Directive from 1996). To control the emission reduction objectives for ozone and its precursors a monitoring obligation has been set up under Directive 2002/3/EC covering the 30 most concerning NMVOC. 2,4,4-trimethylpentene is not included.

Since the problem of NMVOC is addressed by existing EU legislation and taking into consideration that 2,4,4-trimethylpentene from production and processing only contributes to 0.004% of total NMVOC-emissions there is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (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.

3.3.3 Terrestrial compartment

Releases into the terrestrial compartment only occur from atmospherical deposition. Since no ecotoxicological data for suitable organisms are available the calculation is based on the **$PNEC_{soil}$ of 9.27 µg/kg (wwt)** estimated by using Equilibrium Partitioning method and the calculated soil concentrations (see chapter 3.1.5).

All PEC/PNEC ratios are far below 1. Therefore conclusion ii has to be drawn.

Conclusion (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.

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

The log Kow-value indicates a potential for bioaccumulation of 2,4,4-trimethylpentene (see chapter 3.1.3.3). A bioaccumulation study on fish resulted in a bioconcentration factor of 868. For the aquatic food chain a PEC_{oral} for fish was calculated (see 3.1.7). Comparison with the **$PNEC_{oral}$ of 10 mg/kg_{food}** results in the following ratios:

Table 3.24 PEC/PNEC-ratios for secondary poisoning

Company	PEC oral/PNEC oral
Prod.Site A	0.9
Prod.Site B	**3.1**
Proc.Site A	< 0.1
Proc.Site B	< 0.1
Proc.Site C	**8.2**
Proc.Site D	< 0.1

Proc.Site E	0.5
Proc.Site F	0.6
Proc.Site G	< 0.1
Proc.Site H	< 0.1
Proc.Site I	0.2
Proc.Site J	< 0.1
Proc.Site K	< 0.1
Proc.Site L	< 0.1
Proc.Site M	< 0.1
Proc.Site N	< 0.1
Proc.Site O	< 0.1
Proc.Site P	< 0.1
Proc.Site Q	< 0.1
Proc.Site R	0.5
Proc.Site S	< 0.1
Proc.Site T	< 0.1
Proc.Site U	0.1
Proc.Site V	< 0.1
Proc.Site W	0.4
Proc.Site X	0.1
Proc.Site Y	< 0.1
Others	< 0.1

Beside two sites the PEC/PNEC ratio is < 1 indicating no risk for the food chain. Therefore conclusion ii has to be drawn for these sites.

Conclusion (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.

For production site B and processing site C a PEC/PNEC ratio > 1 was calculated. The ratio are 3.1 (Prod.Site B) and 8.2 (Proc.Site C). For Proc.Site C the underlying PEC_{local}(water) was calculated according to the TGD, table A 3.3 and site specific data about the effluent flow and the receiving water flow. For production site B the PEC was calculated according to the ESD IC 3. Due to the uncertainty of the underlying PEC values the assessment of secondary poisoning should be revised based on site-specific exposure data.

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

3.3.5 PBT-assessment

The following table shows the criteria as defined in the TGD to identify PBT/vPvB substances, and the values relevant for 2,4,4-trimethylpentene.

Table 3.25 Data for 2,4,4-trimethylpentene and PBT/vPvB criteria according to TGD

Criterion	PBT-criteria	vPvB-criteria	2,4,4-trimethylpentne
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) No data from simulation studies available.
B	BCF > 2000	BCF > 5000	BCF (fish): 868
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	No chronic NOEC available

2,4,4-trimethylpentene needs to be considered as non biodegradable in the aquatic compartment. Hence, the screening criterion for persistency is fulfilled. However, due to the high vapour pressure, the substance is expected to evaporate from the water phase into the air and hence, is not persistent at least in the aqueous compartment.

The LogKOW of up to 5.0 indicates for a high bioaccumulation portential. The screening criteria for bioaccumulative is fulfilled. However, the highest measured BCF in fish is 868 indicating that bioconcentration in fish is not as high as expected by the LogKOW. With regarnd to the PBT-criteria of the TGD, the criterion for bioaccumulative (“B”) is not fulfilled

Based on the available BCF, it can be concluded that 2,4,4-trimethylpentene does not meet the PBT or vPvB criteria. With respect to the PBT-assessment, a conclusion (ii) can be drawn.

Conclusion (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.

According to the information from industry no sites producing or processing 2,4,4-trimethylpentene are located near the sea. Therefore a marine risk assessment was not conducted.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

2,4,4-Trimethylpentene is primarily used as a chemical intermediate which is further processed to aldehydes, alkyl phenols or polymers. A small portion (according to the Danish Product Register, < 1 t/a) of 2,4,4-trimethylpentene is used in the metal industry for stabilising degreasing agents containing trichloroethylene. The average concentration of 2,4,4-trimethylpentene in degreasing agents is 0.2 % w/w. Otherwise, the concentration of 2,4,4-trimethylpentene in other products is < 0.1 %, most often as a monomer residue in some of (epoxybased) 2-component systems (Danish Product Register, 2003).

For workers the inhalation and dermal routes of exposure are likely to occur.

4.1.1.2 Occupational exposure

Industrial activities using 2,4,4-trimethylpentene present opportunities for occupational exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

Occupational exposure limits have not been established.

The relevant occupational exposure scenario is:
- Production and further processing of 2,4,4-trimethylpentene in the large-scale chemical industry.

On account of the low concentration of 2,4,4-trimethylpentene in degreasing agents and assuming a very low concentration of residual 2,4,4-trimethylpentene in polymers, exposure scenarios in the context of producing and handling these materials are regarded to be of minor relevance. Therefore they are not discussed within the framework of this exposure assessment.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90^{th} or 95^{th} percentiles are derives as representing reasonable worst case situations.

If available, only data measured later than 1990 are used in exposure assessment. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data are not available, model estimates are used.

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.

Within the framework of existing substances there is an agreement between the EU member states, 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 indicating that, for a certain scenario, gloves are a widely accepted protection 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 a criterion. For most downstream uses it is commonly known that gloves are not generally worn. In this cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is usually used for assessing dermal exposure.

4.1.1.2.1 Production and further processing as a chemical intermediate

The production and further processing of 2,4,4-trimethylpentene mainly take place continuously in closed systems. The production is based on a direct oligomerisation over a fixed catalytic bed. The product is separated by distillation. Another industrially applied method for the production of 2,4,4-trimethylpentene is a two step synthesis using sulphuric acid. The first step takes place at about 700 kPa and 20 – 35 °C and the second step at lower pressure and a temperature between 100 – 130 °C. The product is always purified by distillation.
The substance as such is placed on the market with diisobutylene-purities varying from 90 % to 97 %.

According to information provided by a manufacturer exposure associated with transporting the chemical could result from loading, unloading and drumming operations. The delivered 2,4,4-trimethylpentene is filled into tanks (type is not known) by means of a gas displacement device. In-company transport to the point of dispatch is via closed pipelines.

Detailed information concerning the further processing of 2,4,4-trimethylpentene to polymers or regarding the alkylation of phenol is not available. The reaction to aldehydes occurs at 20 000 – 30 000 kPa and 170 °C in a continuous process in closed systems.

Since 2,4,4-trimethylpentene is highly flammable (R 11) it is to be expected that high exposure levels at the workplaces are avoided to a large extent.

In the area of production and further processing of the substance, exposure is possible during sampling, filling operations, coupling and uncoupling transfer lines, repair, cleaning, and maintenance works. Within the large-scale chemical industry high standards of control are normally practised even if the containment may be breached, e.g. during maintenance and taking of samples. Inhalation exposure in other areas is normally minimised by technical equipment (e.g. special designed filling stations, local exhaust ventilation).

Inhalation Exposure

Workplace measurements

Table 4.1.1.2.1: 2,4,4-Trimethylpentene exposures at workplaces during production and further processing

Job category / activities	Years of measurement	Number of samples	Range of measurement data [mg/m^3]	Geometric mean [mg/m^3]	95th percentile [mg/m^3]	Duration / frequency
8h time weighted average (production)						
Operator	1998	32 (p)	0.05 – 7.9	0.4	5	-/-
Different points of production unit	1998	6	0.7 – 9.9	3.6	9	-/-
8h time weighted average (further processing)						
Near feed pumps in plant	1998	2	2.1, 1.3	-	-	-/-
Production	1979 - 1995	93	0 - 2.3 [1)]	0.3 [1)]	-	-/-
Filling/storage	1979 - 1995	11	0 – 0.5 [1)]	0.2 [1)]	-	-/-
Laboratory	1979 - 1995	29	0 – 2.6 [1)]	0.2 [1)]	-	-/-
Different workplaces	1979 - 1995	16	0 – 1.5 [1)]	0.2 [1)]	-	-/-
Pilot plant	1979 - 1995	6	0 – 0.5 [1)]	0.3 [1)]	-	-/-
Short term measurements (further processing)						
During maintenance	1998	2 (p)	10, 21	-	-	6 min/-
Uncoupling after unloading	1998	1 (p)	19	-	-	25 min/-
Sampling and coupling before unloading	1998	1 (p)	90	-	-	46 min/-

(p): personal sampling
[1)] conversion factor for ml/m^3 to mg/m^3 is 4.65

One producer stated that the exposure level for all organic hydrocarbons during production is below 1 mg/m^3.

Another company explained, that during further processing measurements of VOC (volatile organic compounds) were carried out. From these data the exposure level of 2,4,4-trimethylpentene was estimated to be 10.5 mg/m^3.

According to information provided by two producers for the purpose of measuring 2,4,4-trimethylpentene concentration in workplace air, the substance is adsorbed to activated charcoal and detected gas-chromatographically. Due to the measurement method and the sampling strategy applied, the available measurement results submitted by 3 a majority of manufacturers (table 4.1.1.2.1) are regarded as valid.

According to information provided by two manufacturers in the production and further processing of 2,4,4-trimethylpentene 95 and 20 workers, respectively, are employed. Workers normally use personal protection equipment (PPE, gloves, eye glasses).

Based on the measurement results a 8h-TWA of 5 mg/m^3 is regarded as representing a reasonable worst case situation. This exposure level is derived on the basis of personal sampling. Short-term exposures up to 90 mg/m^3 (19.35 ml/m^3) are possible during sampling and coupling before unloading.

Conclusions

Inhalation exposure has to be assessed for production and further processing of 2,4,4-trimethylpentene in areas with high levels of protection, e.g. in the large-scale chemical industry. Inhalation exposure may occur during filling, drumming, sampling and coupling and uncoupling transfer lines, as well as cleaning, maintenance and repair work.

For the assessment of health risks for daily inhalation exposure during production and further processing a 8h-TWA of 5 mg/m^3 (95th percentile) should be taken to represent a reasonable worst case situation. Short-term exposures up to 90 mg/m^3 (46 min) are possible during sampling and coupling before unloading.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to 2,4,4-trimethylpentene are assumed to be daily and for the entire length of shift.

Dermal exposure

Dermal exposure during the production and further processing of substances may occur during activities like drumming, sampling, cleaning, maintenance, coupling and uncoupling transfer lines and repair. 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 210 cm^2 (palms of hands) the model yields an exposure level of 21 - 210 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 in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material of the gloves with regard to the permeation properties of the substance. For 2,4,4-trimethylpentene, importers and producers provide only limited information. Only one of three producers and importers recommends suitable gloves tested according to EN 374. Taking the lack of information into account for assessing dermal exposure during the production and further processing, the use of unsuitable gloves providing only limited protection is presupposed. Since the extent of protection cannot be estimated, actual dermal exposure is assessed to be identical to the potential exposure assessed for the unprotected worker (21 – 210 mg/person/day). The higher level is regarded as representing the reasonable worst case.

Conclusions

In the area of production and further processing of 2,4,4-trimethylpentene, daily dermal exposure is assessed to be 210 mg/person/day (EASE).

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

On account of the high vapour pressure of 2,4,4-trimethylpentene (5.8 kPa), the resulting retention time of the substance on the gloves or the skin is shortened and lower levels of dermal exposure than the estimated ones are to be expected. In appendix A1 the calculation of the evaporation time of the pure substance is described. For 2,4,4-trimethylpentene with the EASE estimate of 1 mg/cm^2, an evaporation time of 12 seconds (T = 30°C) is calculated. For 2,4,4-trimethylpentene on the gloves, an assumed temperature of 20°C leads to an evaporation time of 19 seconds. An evaporation time of 15 seconds should be taken as an order of magnitude, since it is not known in how far the interaction of the skin with the substance influences the evaporation time.

4.1.1.2.2 Summary

Based on the available information, the exposure assessment reveals that handling of 2,4,4-trimethylpentene during production and further processing is the main source for occupational exposure.

On account of the low concentration of 2,4,4-trimethylpentene in degreasing agents and assuming a very low concentration of residual 2,4,4-trimethylpentene in polymers (< 0.1 %), exposure scenarios in the context of producing and handling these materials are regarded to be of minor relevance. Therefore they are not discussed within the framework of this exposure assessment.

The relevant inhalation and dermal exposure levels are given in tables 4.1.1.2.2 A and 4.1.1.2.2 B.

For the large-scale chemical industry, it is assumed that the production and further processing of 2,4,4-trimethylpentene is mainly performed in closed systems. Exposure occurs if the closed system is breached.

Dermal exposure is assessed for daily activities and for occasional cleaning and maintenance of the plant.

Table 4.1.1.2.2 A: Summary of inhalation exposure data of 2,4,4trimethylpentene 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**
Production and further processing								
Production and further processing in the large-scale chemical industry	vapour (liquid)	filling, sampling, cleaning, repair, maintenance, coupling transfer lines	shift length	daily	5	95th percentile	90 (45 min)	highest measurement result

Table 4.1.1.2.2 B: Summary of dermal exposure data of 2,4,4-trimethylpentene 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)**
Production and further processing								
Production and further processing	liquid	filling, sampling, cleaning, repair, maintenance, coupling transfer lines	daily	intermittent	0.1 – 1	210	210 [2)]	EASE (unsuitable gloves)

[1)] contact level according to the EASE model
[2)] Dermal exposure is reduced due to the fast evaporation of the substance

4.1.1.3 Consumer exposure

There is no evidence available on the use of 2,4,4-trimethylpentene in consumer products (GIFAS, Federal Institute for Risk Assessment (formerly BgVV), 1998), hence it is concluded that consumer exposure does not exist.

4.1.1.4 Humans exposed via the environment

According to Appendix VII of chapter 2 of the TGD. the indirect exposure to humans via the environment. i.e. through food. drinking water and air is estimated.

As a worst case scenario. the maximum intake due to exposure in the vicinity of the processing site is calculated. This is compared to an average intake due to exposure via the regional background concentration.

The following input parameters were used for the calculations:

parameters	local scenario (PECs)	regional scenario (PECs)
concentration in porewater of agricultural soil	$1.3 \cdot 10^{-6}$ mg/l	$6.59 \cdot 10^{-11}$ mg/l
concentration in porewater of grassland soil	$1.3 \cdot 10^{-6}$ mg/l	$6.59 \cdot 10^{-11}$ mg/l
concentration in agricultural soil	---	$1.19 \cdot 10^{-09}$ mg/kg
concentration in grassland soil	$2.6 \cdot 10^{-5}$ mg/kg	$1.19 \cdot 10^{-09}$ mg/kg
annual averaged concentration in surface water	0.1886 mg/l	$3.1 \cdot 10^{-10}$ mg/l
annual averaged concentration in the atmosphere1	0.043 mg/m^3	$4.47 \cdot 10^{-06}$ mg/m3
concentration in groundwater	$1.3 \cdot 10^{-6}$ mg/l	$6.59 \cdot 10^{-11}$ mg/l

The resulting total daily dose is: DOSEtot = 1.11 [mg • kg_{bw}^{-1} • d^{-1}] (local scenario)

DOSEtot = $9.7 \cdot 10^{-7}$ [mg • kg_{bw}^{-1} • d^{-1}] (regional scenario)

The calculated total doses comprise the following routes:

Route	**% of total dose**	
	local	**regional**
Drinking water	0.36	0.0002
Fish	42.92	0.04
Stem	0.0009	0.07
Root	55.53	0.03
Meat	0.02	0.61
Milk	0.01	0.36
Air	1.16	98.76

The main route of indirect exposure in local scenario is the intake via ingestion of roots whilst main intake route in regional scenario is the inhalation by air.

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

No data are available on 2,4,4-trimethylpentene.

There are no studies available on oral, dermal or inhalative absorption. From the physico-chemical data (log Pow 5.0, water solubility 1.8 mg/l, molecular weight 112 g/mol, vapour pressure 57.9 hPa at 25°C) the substance shows a good oral and dermal bioavailability. It is proposed to take forward for the risk characterisation values of 100% for oral, dermal and inhalative absorption.

Results from studies on structurally similar compounds

In vitro studies on short chain olefins (n-1-octene, n-4-octene, and 3-ethyl-2-pentene) with rat liver microsomes demonstrated the conversion of olefins to diols via epoxide intermediates (Maynert et al., 1970). Similar reactions can be assumed for 2,4,4-trimethylpentene although the quantitative extent remains to be determined.

4.1.2.2 Acute toxicity

Animal data:

Oral

The acute oral toxicity of 2,4,4-trimethylpentene has proven to be low for rats:

A LD50 value > 2 000 mg/kg bw resulted in a study according to OECD guideline 401 (1987) / ECC guideline B.1 with diisobutene (Shell Chemicals Limited), purity 95.19%. In this study the acute oral toxicity of commercial 2,4,4-trimethylpentene was investigated in a group of 5 male and 5 female rats at a dosage of 2 000 mg/kg bw. The animals were starved overnight prior to dosing. The test material was administered as a constant volume-dosage of 10 ml/kg in maize oil. There were no deaths and no reaction to treatment. All animals achieved anticipated body weight gains and necropsy findings were unremarkable (Huntingdon Life Sciences, 1996a).

Male Wistar rats (15/group; weight 160-210 g) were treated orally with doses of 250, 500, 1 000 or 2 500 mg/kg bw (probably of the undiluted substance). The tested substance was a mixture of C8 olefins (approx. 75% 2,4,4-Trimethylpentene-1 and approx. 15% 2,4,4-Trimethlypentene-2). There were no mortalities. A transient reduction in motor activity was noted at dose levels of 1 000 and 2 500 mg/kg bw. A group of 30 male Wistar rats (weight 160-210 g) was treated daily with increasing doses of the substance (200 mg/kg at day 1, 300 mg/kg at day 2, 450 mg/kg at day 3, 675 mg/kg at day 4, 1 015 mg/kg at day 5) and were observed for 7 days after the last treatment. There were no mortalities. Clinical signs (not specified) were noted after day 3 (Bayer AG, 1972).

A study with another commercial octene (Neodene 8, a linear alpha olefin (SHOP, CAS No. 111-66-0), containing > 98.5 % octene) resulted in an oral LD50 value > 3 550 mg/kg bw (5 ml undiluted substance), using a method not specified, but conforming to EEC B.1. limit test: Five male and 5 female rats were treated with 5 ml/kg undiluted Neodene 8 (3.55 g/kg assuming a density of 710 kg/m^3), while 5/5 control animals received 5 ml/kg deionised water. None of the animals died as a result of treatment; body weight gain was not significantly affected. The main clinical signs of intoxication were manifest within 1 hour and were hunched posture, unsteady stance, tiptoe gait and hypoactivity, less commonly polyuria and stools with mucous were observed. Necropsy revealed no treatment related changes (Shell toxbase, 1993a).

Inhalation

Wistar rats (weight 160-210 g) were exposed for 4 hours (whole body exposure) to 6 different mist concentrations ranging from 7.6 to 44.0 mg/l. The substance was a mixture of C8 olefins (approx. 75% 2,4,4-Trimethylpentene-1 and approx. 15% 2,4,4-Trimethlypentene-2). Twenty male and 20 female rats were used in each group/concentration. Mortality was noted starting at concentrations of 21 – 25 mg/l and the LC 50 was 31.5 mg/l (6 870 ppm) for male rats and 30.0 mg/l (6 540 ppm) for female rats. Exposure (concentration 21.6 mg/l; 4 900 ppm) of 10 male and ten female Wistar rats for 4 hours/day and on five consecutive days resulted in death in 3/10 males and 2/10 females. Animals died within 24 hours. Clinical signs in surviving animals consisted of convulsions, followed by sedation and respiratory distress (Bayer AG, 1992).

A LC50 value of 36.87 mg/l/4 hours (8 050 ppm) was obtained with commercial C8 linear alpha olefin, containing > 98.5 % octene (CAS No. 111-66-0). Ten male rats per group were exposed for 4 hours to concentrations ranging from 6 050 - 11 580 ppm and for 1 hour to the saturated vapors (19 110 ppm, 87.5 mg/l). All deaths occurred within the exposure period. 9/10 rats died during a 1-hour exposure to the saturated vapors (abstract, no further information) (Shell toxbase, 1993a).

In a compilation of all toxicological information on C6-C28 olefins present in the year 1993 the authors stated that aspiration is a significant hazard with C6-C14 olefins (Shell toxbase, 1993a).

Dermal

For 2,4,4-trimethylpentene dermal LD50 values > 2 000 mg/kg bw were determined in the rat. A study in the rabbit is also available as supporting information on a C8 linear alpha olefin, CAS No. 111-66-0.

In a study according to OECD guideline 402 (1987) / ECC guideline B3. with diisobutene (Shell Chemicals Limited), purity 95.19 %, the acute dermal toxicity of commercial 2,4,4-trimethylpentene was investigated in a group of 5 male and 5 female rats. The undiluted test material was applied to the closely-clipped dorsum of each animal at a dosage of 2 000 mg/kg bw, and was covered by an occlusive dressing for 24 hours. None of the animals died. Systemic signs of treatment were restricted to vocalization in all animals 15 minutes after

application of the test material, local signs of reaction comprised very slight or well defined erythema and slight exfoliation. All animals were overtly normal by day 11. The animals achieved anticipated body weight gains; necropsy revealed no significant macroscopic lesions (Huntingdon Life Sciences, 1996b).

In a study using a method not specified but conforming to EEC B.3., a dermal LD50 > 2 000 mg/kg bw was detected for Neodene 8 linear alpha olefin (SHOP, CAS No. 111-66-0), containing > 98.5 % octene. Intact and abraded skin of four male and 4 female Albino rabbits were treated with 2 000 mg/kg neat olefin for 24 hours under occlusive exposure. Control animals received 2 ml/kg deionised water. There were no clinical signs or mortalities as a result of exposure to this product. Skin irritation was assessed at 24 hours and on day 14. Body weight gain was unaffected by treatment. Moderate erythema and oedema was observed at 24 hours and some skin irritation was still evident at 14 days. Necropsy revealed no treatment related changes other than dermal irritation (Shell toxbase, 1993a).

Human data:

No data available.

Conclusion:

Human data on acute toxicity of 2,4,4-trimethylpentene are not available. In animal studies the acute toxicity of this olefin has proven to be low for the oral, dermal and inhalation routes of exposure with oral LD50 values > 2 000 mg/kg for rats, an inhalation LC50 value for rats of 30 000 mg/m^3/4 hours and dermal LD50 values for rats and for rabbits > 2 000 mg/kg. Thus, R phrases according to EU regulations labelling for acute oral, dermal or inhalation toxicity are not appropriate. In compilation of all toxicological information on C6-C28 olefins it is stated that aspiration may be a hazard with C6-C14 olefins. Therefore, R65 "May cause lung damage if swallowed" is proposed.

4.1.2.3 Irritation

4.1.2.4 Corrosivity

Animal data:

The ears of two white New Zealand rabbits were treated twice daily for 5 consecutive days with an unspecified amount of a mixture of C8 olefins (approx. 75% 2,4,4-Trimethylpentene-1 and approx. 15% 2,4,4-Trimethlypentene-2). A transient reddening of the treated skin sites was noted. No other information was given (Bayer AG, 1972).

In Draize tests with rabbits 2,4,4-trimethylpentene and isomers demonstrated moderate skin irritation after a 4-hours skin contact and moderate to severe skin irritation after a 24-hours skin contact:

The potential of diisobutene, purity 95.19 %, to cause inflammatory or corrosive changes upon first contact with skin was assessed in a Draize test according to OECD guideline 404 (1992) / ECC guideline B4 by semi-occluded application of 0.5 ml of the test material to the closely-clipped dorsa of 3 albino rabbits for 4 hours. Dermal reactions were assessed 1, 24, 48 and 72 hours after removal of the dressings and on days 7, 10, 13 and 16. No oedema was observed. Very slight or well defined erythema (mean scores 24 h/48 h/72 h resulted in 1.3/1.3/2) were apparent at the test site of all animals during the first 7 days after bandage removal. Eschar formation was apparent at all test sites during the first 72 hours and exfoliation was evident from day 7-13 in all animals persisting until termination on day 16 in one case (Huntingdon Life Sciences, 1996c).

In a compilation of toxicological test data carried out by the Shell Oil Company short abstracts of the results of a great number of toxicological tests with several linear alpha olefins of 6-18 carbon atoms are listed. None of these test reports is available. Tests with C8-olefins refer to substances mostly called "SHOP alpha olefin C8" or "Neodene 8 alpha olefin". Shop alpha olefin C8 (CAS Nr. 111-66-0) is reported to contain > 98.5% of "C8 octene" which consists of > 95.5% "alpha olefins" and < 3.5% "branched and beta olefins". The results of skin irritation testing are summarised as to be classified as irritant according to EEC/OECD criteria.

In a Draize test using Neodene 8 linear alpha olefin (SHOP), containing > 98.5 % octene, 3 male and 3 female Albino rabbits were each exposed to 0.5 ml of the undiluted substance for a 24-hours covered exposure to intact and abraded skin. The application site was scored for erythema and oedema immediately after removal of the dressing and at 72 hours. The individual scores were summed and used to calculate a group primary irritation index which was 3.38 and indicated a classification of moderate irritant. Some of the erythema scores were classified as 0 as the skin was blanched white, not reddened. Maximum erythema scores were 4 at both 24 and 72 hours. Irritation had increased at 72 hours. The animals were observed up to 7 days and the scores at this time would indicate a classification of severe irritation (only an abstract of the study report given, no further information) (Shell toxbase, 1993a).

Slight eye irritation, not to be labelled according to EU regulations was determined in a Draize eye test according to OECD guideline 405 (1987) / ECC guideline B.5. with diisobutene , purity 95.19 %: The potential of this commercial grade 2,4,4-trimethylpentene to cause damage to the conjunctivae, iris or cornea was assessed in 3 Albino rabbits, each subjected to a single ocular instillation of 0.1 ml of the test material. Ocular reactions were assessed 1, 24, 48 and 72 hours after treatment and on day 8. No corneal lesions and no conjunctival chemosis were noted throughout the study; very slight conjunctivitis was apparent in the treated eye of each animal during the first 72 hours after instillation (mean scores for conjunctival redness 24 h/48 h/72 h resulted in 1/1/0.7); iritis was evident in one animal at the 1-hour assessment. All animals were overtly normal on day 8. Instillation of the test material caused a slight pain response (Huntingdon Life Sciences, 1996d).

Human data:

Three volunteers (2 women, 1 man) were exposed for 5 minutes to a mixture of C8-olefins (approx. 75% 2.4.4-Trimethylpentene-1 and approx. 15% 2.4.4-Trimethylpentene-2). A substance concentration of 465 mg/m^3 (100 ppm) had irritating effects on the mucosa of nose and throat. A substance concentration of 279 mg/m^3 (60 ppm) had no irritating effects but the odour was distinctly smelled and a substance concentration of 46.5 mg/m^3 (10 ppm) was barely smelled (Bayer AG, 1972).

Conclusion:

Human data on local irritation of 2,4,4-trimethylpentene showed effects on the mucosa of nose and throat at a concentration of 465 mg/m³. In Draize tests with rabbits according to EU test guidelines 2,4,4-trimethylpentene demonstrated mild skin irritation after a 4-hours semi-occluded application and mild eye irritation after instillation into the conjunctival sac. The lesions caused after 4 h contact with skin - increasing irritation, eschar formation and exfoliation - point to defatting as main hazardous effect. However, in a compilation of unpublished toxicological test data carried out by the Shell Oil Company, linear alpha olefins containing 8 carbon atoms (CAS No. 111-66-0) are reported to cause skin irritation at a level that requires to be classified as irritant according to EEC/OECD criteria. On the basis of this overall statement of a producer of C8 olefins we propose to classify 2,4,4-Trimethylpentene as "irritant" and label with R38.

4.1.2.5 Sensitisation

Animal data:

In a Magnusson Kligman test 20 test and 10 control guinea pigs of the Dunkin-Hartley strain were used. The groups were evenly divided by sex. Test animals received an intradermal injection of a 50 % 2,4,4.-trimethylpentene (purity: 95.15%) in paraffin oil followed by topical treatment at day 7 with the undiluted test substance. Challenge was conducted in test and control animals with a 75 % and 30 % substance formulation in paraffin oil as for intradermal induction. A significant dermal response (a reaction more marked than the most severe among the control animals) were observed in 3/20 test animals following challenge application of the 75% substance formulation. Grade 1 erythema was noted in 6/20 test and in 3/10 control animals. In 3/20 test animals eschar formation and in 12/20 test animals exfoliation were evident. Thus, the overall positive response rate was 15% (3/20) following application of a 75% substance concentration. A 30% substance formulation caused slight erythema in 3/20 test and in 1/10 control animals. The 75 % substance formulation gave rise to slight erythema in test and control animals at a rate of 30 % in both groups. But in 3/20 (15 %) of the test animals the reaction was more severe (severe erythema) than in controls. Thus 15 % of the test animals exhibited a sensitizing reaction. The 30 % substance formulation resulted in slight erythema in 1/10 (10 %) of the control animals and in 6/20 (30 %) of the test animals. This is considered not to be the result of an immunological reaction (Shell Chemicals Ltd. UK & EC Erdölchemie GmbH, 1997).

Additional data refer to a Buehler test that is mentioned within a compilation of all toxicological information on C6-C28 olefins (Shell toxbase, 1993a). However, the short abstract does not allow proper assessment of that information.

Animal data on sensitization by inhalation are not available.

Human data:

Human data on sensitization by skin contact and after inhalation are not available.

Conclusion:

Human data on sensitization by skin contact or after inhalation are not available. Also animal data on inhalation sensitization are not available. Data on a skin sensitization test according to OECD test guideline 406 are available to allow a judgement on the skin sensitizing properties of the substance. In a Magnusson Kligman test a significant dermal response (a reaction more marked than the most severe among the control animals) were observed in 3/20 test animals following challenge application of the 75% substance formulation. This weak incidence, however, does not result in classification and labelling as sensitizing.

In addition, a Buehler test is mentioned by industry but the short abstract given does not allow an assessment of the skin sensitizing properties of the substance.

4.1.2.6 Repeated dose toxicity

Animal studies

Oral

28-day study (rat)

In a valid 28-day oral toxicity study according to OECD TG 407 (revised 1995) Sprague-Dawley CD-1 rats were administered by gavage with 2,4,4-trimethylpentene (50:50 mixture of two original batches of 2,4,4-trimethyl pentene, Batch No. R11 supplied by Shell Chemicals UK Ltd. and Batch No. 155833 supplied by EC Erdölchemie GmbH, purity 99.1%) daily for 28 days (Huntingdon Life Science, 1997a). Groups of rats (5/sex/group) received 2,4,4-trimethylpentene at dosages of 0, 100, 300, 1 000 mg/kg bw/day. For microscopy the tissue samples were stained with hematoxylin and eosin. There were no special staining for visualization of possible hyaline droplets in renal proximal tubule cells, and furthermore, no specific immunohistochemical detection for the presence of α_{2u}-Globulin in the hyaline droplets. No animal died. Brown staining of the dorsal and ventral fur was observed in males and females receiving 1 000 mg/kg bw/day. Females at this dosage also showed an ungroomed appearance. Salivation after administration occurred on isolated occasions in animals receiving 1 000 mg/kg bw/day. Slight increases in weight gain, food

intake and food conversion efficiency were recorded for females receiving 1 000 mg/kg bw/day. A slight increase in food intake was also recorded for males receiving 1 000 mg/kg bw/day and a slightly increased bodyweight gain and a slightly higher food conversion efficiency were recorded for females receiving 300 mg/kg bw/day. Open field observations indicated yellow/brown staining of the coat from week 2 in males and females receiving 1 000 mg/kg bw/day. No treatment-related changes were identified in the sensory reactivity tests on grip strength and motor activity measurements performed in week 4. There were no toxicologically significant haematological findings at the end of the study. The cellularity and composition of the bone marrow was unaffected by treatment with 2,4,4-trimethylpentene. Blood chemistry investigations during week 4 indicated, when compared with controls, decreased plasma glucose concentrations in females receiving 1 000 mg/kg bw/day, increased total plasma protein and chemical albumin concentrations in males receiving 1 000 mg/kg bw/day and high plasma urea concentrations in males receiving 1 000 mg/kg bw/day but lower urea concentration in females receiving 300 or 1 000 mg/kg bw/day. Compared to controls, absolute and bodyweight-relative kidney weights were significantly higher in males given
1 000 mg/kg bw/day and absolute and bodyweight-relative liver weights were significantly higher in males and females given 1 000 mg/kg bw/day. There were no treatment-related macroscopic or histopathological findings after 4 weeks of treatment.

The liver and kidneys were identified as target organs. The absolute and relative liver weights of male and female rats given 1 000 mg/kg bw/day were significantly increased in comparison with the controls but there was no associated histopathological change. Variations in plasma protein and glucose concentrations may be due to an alteration in liver metabolism following treatment with 2,4,4-trimtethylpentene. Males receiving 1 000 mg/kg bw/day showed a clear increase in kidney weight when compared with the controls but this was also not associated with any histopathological change. The increased urea concentration may be related to a minor alteration in renal function. The NOAEL in this study was considered to be 300 mg/kg bw/day derived from the effect on the liver (Huntingdon Life Science, 1997a).

In a further oral study which was not conducted in accordance with the current guidelines (only summary without detailed information; no GLP, no data on number of animals per sex, and no details on the methods used and the results) a mixture of 75 % 2,4,4-trimethylpentene-1 and approx. 15 % 2,4,4-trimethylpentene-2 was administered to male Wistar rats in increasing doses for 5 days (1. day: 200; 2. day: 300; 3. day: 450; 4. day: 675; 5. day: 1 015 mg/kg bw) followed by one week recovery period. There were no deaths. At doses ≥ 450 mg/kg bw all animals showed symptoms (not specified); no more data available. A NOAEL could not be derived from this study (Bayer AG, 1972).

Data from a reproductive developmental screening test according to OECD TG 421 investigating effects of oral administration of 2,4,4-trimethylpentene in Sprague Dawley CD-1 rats were included to supplement information on toxicity to repeated exposure of 2,4,4-trimethylpentene. In this study 2,4,4-trimethylpentene (50:50 mixture of two original batches of 2,4,4-trimethyl pentene, Batch No. R11 supplied by Shell Chemicals UK Ltd. and Batch No. 155833 supplied by EC Erdölchemie GmbH, purity 99.1%) at 100, 300 and 1 000 mg/kg bw/day was dosed by gavage to groups of 10 males and 10 females for 15 days before mating. Treatment was continued throughout mating, gestation and lactation to day 3 of lactation for females and to termination after approximately 6 weeks of treatment for males (Huntingdon Life Sciences, 1997b). Further information on test procedure and/or reproductive effects are described in section 4.1.2.9.

Relevant treatment-related effects were observed only in male rats. There were nephrotoxic effects, mainly in the cells of the proximal tubuli. After repeated oral exposure of 300 or 1 000 mg/kg bw/day 2,4,4-trimethylpentene an increased absolute and relative kidney weight was exhibited in male rats. Microscopy of the kidneys revealed basophilic cortical tubules at 100 mg/kg bw/day (LOAEL) and above and proteinaceous casts and interstitial inflammatory cells at 300 mg/kg bw/day and above. Females that received 1 000 mg/kg bw/day had slightly elevated kidney weights without relevant microscopic changes.

Kidney sections from this study were subsequently analyzed by immunostaining using mouse anti-α_{2u}-Globulin monoclonal antibodies for the presence of α_{2u}-Globulin which results in the formation of hyaline droplets and kidney toxicity in male (not female) rats. Formalin fixed kidneys (males at 0, 100, 300, 1 000 mg/kg bw/day and females at 0 and 1 000 mg/kg bw/day) were blocked and sectioned for α_{2u}-Globulin staining. Positive control kidney sections from male F344 rats gavaged with d-limonene (150 mg/kg bw/d, over a period of 4 days) were included in this study. The presence of α_{2u}-Globulin was graded from absent (0) through to strong positive staining (grade 4). All slides were randomized before microscopic analyses. These slides were then evaluated by a board certified veterinary pathologist with extensive experience in α_{2u}-Globulin nephropathy (Swenberg and Schoonhoven, 2004, unpublished report). This study demonstrated that oral administration of 2,4,4-trimethylpentene at a daily dose of 100, 300 or 1 000 mg/kg bw/day induces α_{2u}-Globulin nephropathy in male Sprague-Dawley CD-1 rats but not in females. A summary of results from this immunohistochemical evaluation is given in the following table 4.1.

Table 4.1: Results of α_{2u}-Globulin immunohistochemistry of male and female rat kidneys from a reproductive developmental screening test (OECD TG 421) on 2,4,4-trimethylpentene (Swenberg and Schoonhoven, 2004, unpublished report)

Exposure group	Negative	Grade 1 minimal	Grade 2 Mild	Grade 3 moderate	Grade 4 Strong	Average grade
Females, vehicle control	10/10	0/10	0/10	0/10	0/10	0
Females, 1 000 mg/kg bw/d	10/10	0/10	0/10	0/10	0/10	0
Males, vehicle control	0/10	8/10	2/10	0/10	0/10	1.2
Males, 100 mg/kg bw/d	0/10	0/10	8/10	2/10	0/10	2.2
Males, 300 mg/kg bw/d	0/10	0/10	1/10	2/10	7/10	3.6
Males, 1 000 mg/kg bw/d	0/10	0/10	0/10	2/10	8/10	3.8

None of the kidney sections from female control rats or treated at 1 000 mg/kg bw/day exhibited any positive staining for α_{2u}-Globulin. In contrast, all male kidneys exhibited positive staining for α_{2u}-Globulin. However, distinct differences were present between control males and males treated with 2,4,4-trimethylpentene. Dose-related increases in the extent of immunostaining were clearly identified. The control males had minimal evidence for α_{2u}-Globulin accumulation, with an average 1.2, while the males given 1 000 mg/kg bw/day had moderate to severe staining, with an average score of 3.8. The average score for the 300 mg/kg bw/day males was 3.6 (range of 2.0 - 4.0) while the 100 mg/kg bw/day males exhibited mild to moderate staining for α_{2u}-Globulin (2.2, range 2.0 - 3.0). Most of the male rats at 300 and 1 000 mg/kg bw/day exhibited severe staining with numerous specific positive staining droplets and multiple granular casts.

So, it was confirmed by immunohistochemistry that the kidney effects observed in male rats in this study are a consequence of formation of α_{2u}-Globulin. The severity of the 2,4,4-trimethylpentene induced α_{2u}-Globulin nephropathy in male rats is moderate to severe which is identical to other chemicals such as demonstrated in numerous studies with d-limonene or for the structurally analogous substance 2,4,4-trimethylpentane and its main metabolite 2,4,4-trimethyl-pentanol. No positive staining for α_{2u}-Globulin was noted in the kidneys of all female rats.

Overall, there was evidence of an increased number and size of hyaline droplets in renal proximal tubule cells of 2,4,4-trimethylpentene-treated male rats at all dosages, increasing in intensity with increasing dose, and that the accumulation protein in the hyaline droplets is α_{2u}-Globulin, and furthermore that daily dose of ≥100 mg/kg bw/day induces α_{2u}-Globulin nephropathy (LOAEL). This was confirmed using immunohistochemical staining with mouse anti-α_{2u}-Globulin monoclonal antibodies (Swenberg and Schoonhoven, 2004, unpublished report). Renal lesions in male rats associated with accumulation of α_{2u}-Globulin is known as a species-specific effect in male rats and not predictive of a similar risk to humans.

Inhalation

There are no valid animal studies with inhalation exposure of 2,4,4-trimethylpentene available.

In an inhalation study without consistence to regular test protocols of current guidelines for subacute inhalation studies (only summary; no GLP; no details on the methods used and the results), Wistar rats (10/sex/group) were exposed to a mixture of 75 % 2,4,4-trimethylpentene-1 and approx. 15 % 2,4,4-trimethylpentene-2 concentrations of 13.2 and 21.6 mg/l for 4 hours/day for 5 days followed by a 2 week recovery period. For the 21.6 mg/l rats, mortality prior to terminal euthanasia in 3/10 males and 2/10 females were exposure related. At 13.2 mg/l, all animals showed symptoms (not specified), no other data. A NOAEC for systemic or local effects could not be derived from this study (Bayer AG, 1972).

Dermal

No repeat dose animal studies were available using the dermal route of exposure of 2,4,4-trimethylpentene.

There were no studies on subchronic and chronic toxicity with oral, inhalation or dermal administration.

Other/further information

- Mechanism of nephrotoxicity in male rats

α_{2u}-Globulin nephropathy is recognized as an important toxicologic syndrome that occurs in male rats without affecting female rats or other species such as dogs, guinea pigs and mice following exposure to a variety of industrial and environmental chemicals. (e.g. unleaded gasoline, branched aliphatic hydrocarbons (e.g. 2,2,4-trimethylpentane), cyclic aliphatic hydrocarbons (e.g. *d*-limonene, isophrone), halogenated aliphatic and aromatic compounds (e.g. tetrachlorethylene, 1,4-dichlorobenzene) and others (Hard et al., 1993). α_{2u}-Globulin nephropathy is characterized by an increase in size and number of protein droplets in the proximal convoluted tubules, granular cast formation at the junction of the proximal tubule and the thin loop of Henle, and scattered cortical tubular epithelial regeneration (Stonard et al., 1985, Short et al., 1986). The protein droplets that accumulate after chemical exposure have been shown to contain α_{2u}-Globulin, a low-molecular-weight protein. Several studies were undertaken to examine the possible relationship between these droplets and α_{2u}-Globulin in normal rats and in rats treated with these chemicals.

Examinations of 15 different hydrocarbon compounds showed that the nephropathy of gasoline was primarily due to the alkane components, and that the nephrotoxic potency of these compounds increases with the degree of branching in the C chain (Halder et al., 1985). Consequently, 2,2,4-trimethylpentane (CAS No. 540-84-1) was selected as a pure compound for studying the mechanism of unleaded gasoline-induced α_{2u}-nephropathy. The following

data presented were received from studies performed with 2,2,4-trimethylpentane a structurally analogous substance to 2,4,4-trimethylpentene.

- Data from structurally analogous substances:

2,2,4-trimethylpentane

The acute or subchronic phase for these nephropathy is characterized by the accumulation of α_{2u}-Globulin in protein droplets (lysosomes), subsequent degeneration and necrosis of individual cells lining the P_2 segment of the proximal tubule. The loss of P_2 segment epithelial cells leads to a restorative hyperplasia of these cells. The extent cell proliferation is dependent upon the dose and number of exposures, the age of the rat, and the period of time elapsed from the last exposure. This effect has been demonstrated in male rats (only in adults), but not in female rats or either sex of mice (Short et al., 1989a,b; Swenberg et al., 1989).

2,2,4-trimethylpentane causes protein droplet accumulation along with a two- to threefold increase in the concentration of the male-rat-specific protein, α_{2u}-Globulin measured in the kidneys of F344 male rats 24 and 48 hr after treated with a single dose of [^{14}C]2,2,4-trimethylpentane (4.4 mmol/kg; 502 mg/kg bw, 2 μCi/mmol) by gavage, but not in female rats. Identification and quantitation of the urinary metabolites of 2,2,4-trimethylpentane showed that both male and female rats metabolize 2,2,4-trimethylpentane via the same pathway and at the similar rate. A marked and consistent difference appeared in the disposition of 2,4,4-trimethyl-2-pentanol. Which is retained in the kidney of male rats. Female rats, however, excreted more conjugates of 2,4,4-trimethyl-2-pentanol in urine than males. 2,4,4-trimethyl-2-pentanol was the major metabolite present in male rat kidney, but was absent in the female rat kidney. The male rat kidney has a specific component, which could be α_{2u}-Globulin, causing a male-rat-specific retention of 2,4,4-trimethyl-2-pentanol (Charbonneau et al., 1987a,b).

Biochemical and histopathologic parameters of nephrotoxicity were measured in groups of male F344 rats (5/group) after a 2-week (5 days/week) oral administration by gavage of 0, or 346 mg/kg 2,2,4-trimethylpentane. The parameters for optical microscopic examination of the kidney used in this evaluation were presence of hyaline droplets in the cytoplasm of proximal cells (azocarmine stain), presence of granular casts within the lumen of the tubules (H & E) and evidence of tubular cell regeneration (H & E). Histopathologic evaluation showed the development of tubular lesions characteristic of the so-called hydrocarbon-induced nephropathy (Gerin et al., 1988). These results on 2,2,4-trimethylpentane were compatible with findings from other repeated dose toxicity studies in male rats (Halder et al., 1985).

Other studies also showed that α_{2u}-Globulin was increased in male kidney in a dose- and time-related fashion, whereas it remained below the limit of detection in female rat kidney (Stonard et al., 1986; Charbonneau et al., 1987a,b). This was demonstrated in studies where the dose of 2,2,4-trimethylpentane was increased from 5 to 50 mg/kg bw/day. The increase in renal α_{2u}-Globulin was 3.5 times greater than in 2,2,4-trimethylpentane equivalents. At 50 mg/kg bw/day severe protein droplet nephropathy and high levels of regenerative hyperplasia were observed in male rats, but it was also noted that a large percentage (~70%) of the α_{2u}-Globulin present in the kidney of male rats is unbound. The remaining, non-absorbed protein was excreted in the urine (Short et al., 1987).

2,2,4-trimethyl-2-pentanol was identified as the major metabolite of 2,2,4-trimethylpentane, a high-affinity ligand for α_{2u}-Globulin (Borghoff et al., 1991), detected in the male rat kidney and was associated with an increase in the renal concentration of α_{2u}-Globulin; this metabolite was not detected in female rat kidney (Charbonneau et al., 1987a). Further studies reproducing time course data of blood and kidney 2,2,4-trimethyl-2-pentanol and renal α_{2u}-Globulin concentrations, suggested that renal accumulation of α_{2u}-Globulin is not simply a consequence of reduced proteolytic degradation but may also involve a transient increase in hepatic α_{2u}-Globulin production (Kohn and Melnick, 1999).

To study the ability of 2,2,4-trimethylpentane to cause the accumulation of α_{2u}-Globulin and renal cell replication, groups of F344 male rats (13 weeks of age) were administered by gavage (5/group) at dosages of 0, 0.95, 3, 6 and 30 mg/kg bw/day for a period of 10 consecutive days. To measure cell replication, rats were exposed to [^{3}H]thymidine continuously over the last 7 days of exposure period. α_{2u}-Globulin was measured in the kidney samples using a an enzyme-linked immunosorbent assay (ELISA) with a mouse monoclonal antibody raised toward purified rat urinary α_{2u}-Globulin. Twenty-four hours after final dose, protein droplet accumulation, α_{2u}-Globulin concentration, and the nuclear labeling index, as a measure of cell replication, were measured in the kidneys of control and treated rats. There were dose-related increases in protein droplet accumulation, α_{2u}-Globulin concentration, and cell replication in the kidneys of rats treated with 2,2,4-trimethylpentane (Borghoff et al., 1992).

Data on olefins

There are data in the Shell toxbase on olefins (C6-C18 or C6-C28) including a C8 linear alpha olefin. Olefins have been tested in a number of inhalation, oral and dermal studies. Data from these investigations have not been evaluated because the studies are too briefly described (published as abstract only). They are, however, included as additional information (Shell toxbase, 1993b, 1993c, 1993d).

Reliability and relevance of these data were uncertain. Inspite of the inadequacies in the cited studies one of them was reported hereafter:

A C8 linear alpha olefin (1-octene) was tested in a 90-day gavage study (similar to OECD TG 408). Groups of 20 male and 20 female rats received a daily gavage administration of 0, 5, 50, or 500 mg/kg bw/day 1-octene (summary report without detailed information). Slight changes indicative of kidney damage were seen only at the high dose level of 500 mg/kg bw/day. The changes included increased kidney weights, decreased urinary volume and unspecified microscopic changes in the kidneys of male rats and increased plasma creatinine in female rats only. The NOAEL for the 13 week (gavage) study with 1-octene was 50 mg/kg bw/day. The kidney is a target organ at higher dose level (Shell toxbase, 1993e).

Summary of repeated dose toxicity data in animals

In a well performed and reported study on 28-day toxicity study of 2,4,4-trimethylpentene the liver and kidneys were identified as target organs. Significantly increased liver weights (absolute and relative) associated with variations in plasma protein (males) and glucose (females) concentrations but without corroborating findings in histology were recorded in male and female rats of the 1 000 mg/kg bw/day groups. In addition, males receiving 1 000 mg/kg bw/day 2,4,4-trimethylpentene showed a clear increase in kidney weight when compared with the controls but this was also not associated with any histopathological change. The increased urea concentration may be related to a minor alteration in renal function. No relevant toxic effect was seen at 300 mg/kg bw/day (Huntingdon Life Science, 1997a).

In an oral reproductive developmental screening test of 2,4,4-trimethylpentene (OECD TG 421), renal lesions (increased kidney weight, and nephropathy induced by a dose-related increased accumulation of α_{2u}-Globulin) were seen in male Sprague Dawley CD-1 rats at doses of ≥100 mg/kg bw/day (LOAEL). This was confirmed by immunohistochemistry staining using mouse anti-α_{2u}-Globulin monoclonal antibodies. The severity of this alteration induced was moderate to severe which was identical to other chemicals such as d-limonene or the close structural analog, 2,2,4-trimethylpentane, which have also been shown to induce moderate to severe α_{2u}-Globulin nephropathy. Females receiving 1 000 mg/kg bw/day had slightly elevated kidney weights without such microscopic changes.

Kidney effects in male rats observed in the reproductive developmental screening test on 2,4,4-trimethylpentene are linked with the accumulation of α_{2u}-Globulin, a low molecular weight protein almost exclusively produced by the male rat, and are, therefore, not relevant to humans. (Huntingdon Life Sciences, 1997b; Swenberg and Schoonhoven, 2004, unpublished report).

There were no other valid data with other routes of exposure than oral to 2,4,4-trimethylpentene.

No observed adverse effect level (NOAEL):

Oral administration

For the purpose of quantitative risk assessment procedures the 28-day rat toxicity study and the reproductive developmental screening test of Huntingdon Life Science (1997a,b) were considered to give the most reliable data on the effect levels of systemic toxicity of 2,4,4-trimethylpentene. Both studies were performed at the same dose levels in Sprague Dawley CD-1 rats.. The 28-day toxicity study was designed to the requirements of the OECD TG 407 (revised 1995). No adverse effect was observed at 100 and 300 mg/kg bw/day 2,4,4-trimethylpentene in both male and female rats in this study, therefore 300 mg/kg bw/day derived from the effect on the liver is considered to be the NOAEL for 2,4,4-trimethylpentene.

The oral reproductive developmental screening test according to OECD TG 421 showed that there is an appreciable difference between male and female rats because nephropathy observed in male rats at all 2,4,4-trimethylpentene treatment groups (LOAEL 100 mg/kg

bw/day). Basophilic cortical tubules were seen in all male rats treated with 2,4,4-trimethylpentene whilst proteinaceous casts and interstitial inflammatory cells were detected only at dosages of 300 or 1 000 mg/kg bw/day. The severity of basophilic cortical tubular changes was more pronounced in males given 300 or 1 000 mg/kg bw/day than those given 100 mg/kg bw/day. α_{2u}-Globulin immunohistochemistry of male and female kidneys from this study demonstrated that the renal lesions seen in male rats in this study are a consequence of the formation of α_{2u}-Globulin (Huntingdon Life Sciences, 1997b; Swenberg and Schoonhoven, 2004, unpublished report).

In the described 4-week oral gavage toxicity study, there was no male nephrotoxicity demonstrated although 2,4,4-trimethylpentene was tested at the same dose levels. In contrast, α_{2u}-Globulin immunohistochemistry of the male kidneys in the reproductive developmental screening test exhibited that 2,4,4-trimethylpentene induces α_{2u}-globulin-associated nephropathy. A possible cause of this difference is that male rats in the reproductive developmental screening test with 2,4,4-trimethylpentene were sexually mature, compared to the pubescent males in the 4-week toxicity study. Because α_{2u}-globulin is not produced in the male rat until after puberty (Short et al., 1989 a,b; Swenberg et al., 1989), the nephrotoxicity would therefore not be expected to be present in the 4-week toxicity study.

In previous evaluations it was considered that humans are not at risk to develop this special type of nephropathy, since they seem to be unable to synthesize α_{2u}-Globulin and the urinary secretion of proteins is in general less than that of the rat. Furthermore, the proteins are either not structurally related to α_{2u}-Globulin or do not bind compounds that bind to α_{2u}-Globulin (Borghoff et al., 1991; Kohn and Melnick, 1999; Swenberg and Lehmann-McKeeman, 1999). Therefore, α_{2u}-Globulin mediated kidney effects in male rats proved in the reproductive developmental screening test with 2,4,4-trimethylpentene by immunohistochemistry staining cannot be regarded as a reliable indicator for the purpose of risk assessment for humans.

So, a NOAEL of 300 mg/kg bw/day based on the effect on the liver in male and female rats from the 28-day oral toxicity study is used as the starting point for risk characterization.

NOAEL

Male and female CD-1 rats (28-day oral (gavage) study) 300 mg/kg bw/day

Inhalation/Dermal application

There are no valid animal inhalation studies of 2,4,4-trimethylpentene available. At present, no studies with dermal administration of 2,4,4-trimethylpentene are available.

Human data:

No data available.

Conclusion/Classification repeated dose studies

The data submitted are acceptable with respect to the basic requirements as specified in Annex VIIA of Directive 67/548/EEC.

Classification and Labeling

No need for classification:

The effects seen in the repeated dose toxicity tests do not justify classification of 2,4,4-trimethylpentene with Xn and R 48 according to the criteria of Directive 93/21/EEC.

4.1.2.7 Mutagenicity

In vitro studies: Bacterial mutation assays

2,4,4-Trimethylpentene (Batch No. 2 was a 50:50 mixture of two original batches of 2,4,4-trimethylpentene - the details of which are as follows: Batch No. R11 supplied by Shell an Batch No. 155833 supplied by Erdölchemie) was examined for mutagenic activity in the *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA in compliance with OECD guideline 471/472 (Shell 1996). The tests were conducted in the absence and presence of Aroclor 1254 induced rat liver S9-mix up to 5 000 µg/plate. No substance induced mutants were observed.

In vitro studies: Cytogenetic test with human lymphocytes

2,4,4-Trimethylpentene (Batch No. 2 was a 50:50 mixture of two original mixtures of 2,4,4-trimethylpentene - the details of which are as follows: Batch No. R11 supplied by Shell an Batch No. 155833 supplied by Erdölchemie) was examined for clastogenic activity with human lymphocytes in compliance with OECD guideline 473 (Shell, 1997). In the absence of S9-mix the test substance was dosed at 5, 10 and 25 µg/ml and the cells were exposed to 2,4,4-trimethylpentene for 20 h and 44 h. In the presence of S9-mix 2,4,4-trimethylpentene was tested at concentrations of 10, 25 and 75 µg/ml and after a three hour exposure the cells were sampled at 20 and 44 h after commencing the treatment. 2,4,4-trimethylpentene did not show increased aberrations excluding gaps under the conditions of test. The highest concentrations scored for chromosomal aberrations resulted only in minor toxicity (based on measurement of mitotic index) but higher concentrations (75 µg/ml without S9-mix; 100 µg/ml with S9-mix) resulted in 100 % reduction of mitosis. The results were verified in independent experiments.

In vivo studies:

No in vivo studies are available.

Conclusion

On the basis of negative results from a bacterial mutation test and a chromosomal aberration test with human lymphocytes in vitro there is no evidence of a genotoxic potential of 2,4,4-trimethylpentene. Furthermore there is no structural alert for genotoxic potential.

4.1.2.8 Carcinogenicity

Animal studies:

No cancer studies on 2,4,4-trimethylpentene are available. Data from mutagenicity give no concern on carcinogenic properties of 2,4,4-trimethylpentene.

Alternative data from a structurally analogous substance 2,2,4-trimethylpentane (CAS No. 540-84-1) will be discussed below.

Investigations in the past have shown that the nephropathy of gasoline was primarily due to the alkane components, and that the nephrotoxic potency of these compounds increases with the degree of alkane branching (Halder et al., 1985). So, 2,2,4-trimethylpentane, one of the most active nephrotoxic components in this mixture, was selected as a pure compound to study the mechanism of renal toxicity induced by unleaded gasoline. The following data were received from selected studies performed with 2,2,4-trimethylpentane as a structurally analogous substance to 2,4,4-trimethylpentene.

Several intermediates of 2,2,4-trimethylpentane metabolism were administered to male F344 rats; and kidney sections were studied for evidence of α_{2u}-Globulin accumulation (Charbonneau et al., 1987a). All chemicals studied, including the carboxylic acid metabolites of 2,2,4-trimethylpentane (Charbonneau et al., 1987a,b), which do not bind to α_{2u}-Globulin, caused accumulation of α_{2u}-Globulin. This observation suggests that binding of α_{2u}-Globulin may not be the only process associated with 2,2,4-trimethylpentane-induced renal accumulation of this protein. But these chemicals do not bind to other members of the $\alpha_{2\mu}$-Globulin superfamily. Furthermore, it has been shown that 2,4,4-trimethyl-2-pentanol, the major metabolite of 2,2,4-trimethylpentane, does not bind specifically to any low molecular weight protein isolated from male human kidney, indicating that there are no proteins constitutively present in human kidney that are similar to α_{2u}-Globulin.

In male F344 rats treated with 2,2,4-trimethylpentane for three weeks, the dose-response curve for renal protein droplet accumulation correlated with that for increased replication of epithelial cells in the P_2 segment of renal proximal tubules (Short et al., 1987).

A large study was designed to evaluate the potential of unleaded gasoline and 2,2,4-trimethylpentane to cause chronic increases in cell proliferation and to promote renal

neoplasia. Cell proliferation was determined within the tree proximal tubule segments of the kidney (P_1, P_2, and P_3) and proximal tubule segments affected by chronic progressive nephrosis in male and female F344 rats exposed to 50 ppm 2,2,4-trimethylpentane for 3 to 50 weeks. No increase in cell proliferation was detected in female rat regardless of site within the nephron, age of the animal, or number of exposures. In contrast, there was an increase in cell turn over (up to 11-fold) in the P_2 segment of male rats that persisted during chronic exposure. This proliferative response closely paralleled the extent and severity of immunhistochemically detectable α_{2u}-Globulin in the P_2 segment. Neither α_{2u}-Globulin nor cytotoxicity was evident in cells of the P_1 or P_2 segment. However, cell proliferation was increased (up to 8-fold) for up to 22 weeks of exposure in P_3 segment. Increased numbers of proximal tubules affected by chronic progressive nephrosis were found in 2,2,4-trimethylpentane exposed males for 22 or 48 weeks, compared to controls. These lesions contained epithelial cells that were highly proliferative (Short et al., 1989a).

Results of a kidney initiation-promotion study on unleaded gasoline and 2,2,4-trimethylpentane have shown an increased number of renal neoplastic lesions classified as atypical cell foci and renal cell tumors. These renal lesions were present in male but not in female F344 rats initiated with 170 ppm N-ethyl-N-hydroxy-ethylnitrosamine given in the drinking water for 2 weeks and subsequently inhalation exposed to 50 ppm 2,2,4-trimethylpentane for 24, or 59 to 61 weeks. The extent of promotion was paralleled the effects of the compound on chronic cell proliferation (Short et al., 1989b).

On the basis of these initiation-promotion studies compelling evidence was provided that 2,2,4-trimethylpentane and other act as renal tubule tumor promoters and that this promoting effect requires α_{2u}-Globulin. Furthermore, there was no elevation of either hyperplastic foci or renal tumors in female rats in the study, emphasizing once again, the male specificity of the renal response to α_{2u}-Globulin accumulation.

- α_{2u}-Globulin:

Association with chemically induced renal toxicity and neoplasia in the male rat

A diverse group of non-genotoxic chemicals has been shown to cause acute renal changes manifested by accumulation of protein, α_{2u}-Globulin, in phagolysosomes of renal proximal tubule cells. α_{2u}-Globulin nephropathy is a syndrome that occurs exclusively in male rat kidney. α_{2u}-Globulin nephropathy syndrome follows from overload of α_{2u}-Globulin that is typically induced by a xenobiotic substance. The protein overload causes renal cell injury, compensatory cell proliferation and ultimately a low but significant incidence of renal tubule tumors (Swenberg and Lehman-McKeeman, 1999). α_{2u}-Globulin, a low molecular weight protein is almost exclusively produced by the male rat. This type of secretion has not been identified in human kidneys or urine (Borghoff et al., 1992). Several chemicals that induce α_{2u}-Globulin nephropathy have been shown to promote both spontaneously and chemically initiated tubule epithelial cells to preneoplastic and/or neoplastic lesions in male rat kidney. In addition, a quantitative relationship between sustained renal-cell proliferation and the promotion of preneoplastic lesions and/or neoplastic lesions has been established, providing support for the conclusion that sustained renal cell proliferation is causally related to the development on renal tumors in male rats (Melnick and Kohn, 1999). The uniqueness of the mechanism for this type of renal carcinogen is further substantiated by the fact that in the

NBC-Black-Reiter rat strain, which lacks hepatic mRNA for α_{2u}-Globulin (Chatterjee et al., 1989), no hyaline droplets or other aspects of renal disease are induced by chemicals which induce hyaline droplets in males of other rat strains (Dietrich and Swenberg, 1991a,b).

Classical renal carcinogens, such as certain nitrosamines, induce renal tubule cancer in rats and mice with high incidence, minimal duration of exposure and clear dose-response relationships. The renal tumors associated with α_{2u}-Globulin nephropathy are also distinguished from classical renal carcinogens in that they show a much longer latency period, requiring at least 18 months of continued dosing, and the chemicals that cause the male-specific renal tumors are not genotoxic (Hard et al., 1993, Swenberg et al., 1989, Swenberg and Lehman-McKeeman, 1999).

The studies described above (cf. also 4.1.2.6.) show that a requisite step in the development of α_{2u}-Globulin nephropathy is the binding of a chemical (or metabolite) to α_{2u}-Globulin. α_{2u}-Globulin is a member of a superfamily of proteins that bind and transport a variety of ligands. Many of these proteins are synthesized in mammalian species, including humans. However, the protein composition of human kidney is very different from that of male rat kidneys and there was no specific binding of α_{2u}-Globulin ligands to human renal protein. Human urinary protein is also predominantly a species of high molecular weight, and there is no protein in human plasma or urine identical to α_{2u}-Globulin. Humans lack the α_{2u}-Globulin that is abundantly secreted by male rats and that is associated with the described species specific form of nephropathy.

The following results of studies cited above indicate that 2,2,4-trimethylpentane fulfils the IARC criteria to identify a carcinogen that acts solely through α_{2u}-Globulin nephropathy (Rice at al., 1999):

- The chemical and its metabolites lack genotoxic activity. The concept that chemicals inducing α_{2u}-Globulin nephropathy cause renal tubule tumors by secondary, non-genotoxic mechanisms is further supported by results of several initiation-promotion studies.

- α_{2u}-Globulin nephropathy is a syndrome that occurs exclusively in male rat kidney.

- α_{2u}-Globulin nephropathy syndrome follows from overload of α_{2u}-Globulin that is typically induced by a xenobiotic substance.

- There is a reversible binding of the chemical or metabolites to α_{2u}-Globulin in the renal proximal tubules.

- The increased proliferative response caused by chemically induced cytotoxicity may be plausible reason for the development of renal tubule tumor in male rats.

Human data:

No data are available.

Conclusion

There are no cancer studies on 2,4,4-trimethylpentene available. Data from mutagenicity testing give no concern on genotoxic properties of the substance.

In a reproductive developmental screening test accumulation of hyaline droplets in kidney tubules was observed in male rats known as a species and sex-specific phenomenon, which results from the excessive accumulation of α_{2u}-Globulin in renal proximal tubular epithelial cells. Results from α_{2u}-Globulin immunohistochemistry of kidneys of male and female rats from this study exhibited that oral administration of 2,4,4-trimethylpentene at daily dose of 100, 300 or 1 000 mg/kg bw/d induces α_{2u}-Globulin nephropathy identical in severity to other chemicals such is demonstrated in several studies with d-limonene or for the structurally analogous substance 2,2,4-trimethylpentane. Numerous studies with 2,2,4-trimethylpentane have been performed to examine the mechanism of inducing renal toxicity and renal cortical tumors in male rats through an α_{2u}-Globulin-associated response. 2,2,4-trimethylpentane is one of the known non-genotoxic chemicals causing α_{2u}-Globulin nephropathy. Biochemical and pathophysiological data from selected studies on 2,2,4-trimethylpentane have shown the major events linked between the α_{2u}-Globulin nephropathy and the carcinogenic outcome such as protein droplets, increased α_{2u}-Globulin, binding to α_{2u}-Globulin, cell proliferation, and initiation/promotion. These data provide convincing evidence to support a linkage between α_{2u}-Globulin nephropathy and renal tubule neoplasia, a mechanism that occurs exclusively in male rats.

Humans lack the α_{2u}-Globulin that is secreted by male rats and that is associated with this species specific form of nephropathy. Therefore, the male rat kidney response to 2,4,4-trimethylpentene is not relevant to human risk assessment. Taking into account the negative mutagenicity data it is concluded that carcinogenicity should not be an endpoint of concern for humans.

4.1.2.9 Toxicity for reproduction

Animal data:

The influence of 2,4,4-trimethylpentene on the F_0 generation gonadal function, mating behaviour, and fertility, and the subsequent performance of the F_1 offspring to postnatal day 4 was assessed in sexually mature male and female rats of the CD strain in a reproductive developmental screening test according to OECD Guideline 421 (Huntingdon Life Sciences, 1997b). The TMP used in this study was a 50:50 mixture of two original batches of 2,4,4-trimethyl pentene (of batch No. R11 supplied by Shell Chemicals UK Ltd and of batch No. 155833 supplied by EC Erdölchemie GmbH). For the purpose of this study 2,4,4-trimethylpentene was administered by oral gavage at dosages of 100, 300 or 1 000 mg/kg bw/day in maize oil at a volume-dosage of 5 mg/kg bw to groups of ten male and ten female rats for 15 days before pairing. Controls received maize oil only. Treatment was continued throughout mating, gestation and lactation to day 3 of lactation for females and to termination after approximately six weeks of treatment for males. All females were permitted to deliver and rear their offspring to postnatal day 4. Control animals received the vehicle, maize oil, throughout the same period.

There was no treatment-related mortality. Treatment-related clinical signs were limited to the high dose group (1 000 mg/kg bw/day) including transient salivation after dosing and brown or yellow coat staining. Body weight gain and food consumption were not affected by

treatment during the entire test period. Examination of parental animals killed at terminal sacrifice revealed that all males and four females of the high dose group had swollen liver lobes, and two males also had large kidneys. The absolute and relative organ weights of livers and kidneys were significantly increased for males that received 300 or 1 000 mg/kg bw/day and for females that received 1 000 mg/kg bw/day. For reproductive organs there were no changes in absolute and relative organs weights even at the high dose level. Histopathological findings that were considered to be related to treatment were observed in the kidneys of all groups of treated males including basophilic cortical tubules at 100 mg/kg bw/day and above and proteinaceous casts and interstitial inflammatory cells at 300 mg/kg bw/day and above. Oestrus cycles, mating performance, fertility, parturition, number of corpora lutea and implantation sites, litter sizes, sex ratio and offspring survival and body weight performance during the first four days of age were not affected by treatment with any dosage of 2,4,4-trimethylpentene. Likewise, macroscopic examinations of the offspring at necropsy on postnatal day 4 revealed no findings which could be ascribed to parental treatment with 2,4,4-trimethylpentene.

Human data:

No data available.

Conclusion

Oral administration of 2,4,4-trimethylpentene to CD rats for 40 to 46 days (during premating, mating, gestation and up to lactation day 4) at dosages of up to 1 000 mg/kg bw/day did not reveal any indications for an impairment of reproductive performance and capability or peri/postnatal viability and performance of offspring up to postnatal day 4 at a screening level. (NOAEL for reprotoxic effects: 1 000 mg/kg bw/d).

4.1.3 Risk characterisation

4.1.3.1 General aspects

No data on the absorption, kinetics, metabolism and distribution of 2,4,4-trimethylpentene are available. From the physico-chemical data (log Pow 5.0, water solubility 1.8 mg/l, molecular weight 112 g/mol, vapour pressure 57.9 hPa at 25°C) the substance shows a good oral and dermal bioavailability. It is proposed to take forward for the risk characterisation values of 100% for oral, dermal and inhalative absorption.

Human data on acute toxicity caused by 2,4,4-trimethylpentene are not available. In animals, acute toxicity by the oral, dermal, and inhalation routes is low as shown from oral LD50 values > 2 000 mg/kg for rats, an inhalation LC50 value for rats of 30 000 mg/m^3/4 hours and dermal LD50 values for rats and for rabbits > 2 000 mg/kg. Aspiration has to be considered as a potential hazard.

Human data showed irritating effects on the mucosa and throat at a concentration of 465 mg/m³. The substance demonstrated mild skin irritation and mild eye irritation in tests with rabbits. Human data on sensitization are not available. In a Magnusson Kligman test 15 % of the test animals showed a positive reaction thus the substance is considered as a weak skin sensitiser.

Oral administration of 2,4,4-trimethylpentene to Sprague-Dawley CD-1 rats at a dosage of 1 000 mg/kg bw/day for four weeks caused significantly increased absolute and relative liver weights in males and females associated with relevant variations in plasma protein and glucose, but without histopathological change, and additionally in males, an increase in kidney weights without corroborative findings in microscopy. An increased urea concentration observed in male rats at 1 000 mg/kg bw/day may indicate an impaired renal function. A NOAEL of 300 mg/kg bw/day for the effect on the liver for both genders was derived from this study. In a reproductive developmental screening test nephrotoxic effects were observed only in male Sprague-Dawley CD-1 rats at ≥100 mg/kg bw/day (LOAEL), mainly in the cells of the proximal tubuli. These renal lesions were induced by a dose-related increased accumulation of α_{2u}-Globulin, a sex- and species-specific phenomenon, confirmed by immunohistochemical analysis of kidney slides using mouse anti-α_{2u}-Globulin monoclonal antibodies. Thus, these findings are not considered to be predictive for similar effects in humans. No valid animal studies with inhalation exposure of 2,4,4-trimethylpentene are available. Repeated dose toxicity via dermal application of 2,4,4-trimethylpentene was not investigated.

There is no information on health effects in humans after repeated exposure.

On the basis of negative results from bacterial mutation tests in S. typhimurium and E. coli and a chromosomal aberration test with human lymphocytes in vitro there is no evidence of a genotoxic potential of 2,4,4-trimethylpentene. No in vivo data are available for mutagenicity.

There are no experimental data on carcinogenicity of 2,4,4-trimethylpentene available. Studies with the structurally analogous substance 2,4,4-trimethylpentane have shown that the formation of renal tubule tumours in male rats is linked through α_{2u}-Globulin nephropathy. Clear dose-response relationships were demonstrated between severity of nephropathy and

renal tubule cancer in male rats in several bioassays. However, this toxicity only occurs in male rats of strains that synthesize α_{2u}-Globulin and not in other species, including humans. In a reproductive developmental screening test with 2,4,4-trimethylpentene dose-related increased incidences of kidney lesions were observed in male, but not in female rats. Results of α_{2u}-Globulin immunohistochemistry in rat kidneys from this study have shown that 2,4,4-trimehtylpentene induces α_{2u}-Globulin nephropathy. Based on the results of this study and the prior research on α_{2u}-Globulin nephropathy, the kidney effects associated with 2,4,4-trimethylpentene-induced α_{2u}-Globulin nephropathy can be considered without relevance to carcinogenicity risk assessment. Taking into account the negative mutagenicity data it is concluded that carcinogenicity should not be an endpoint of concern for humans.

Oral administration of 2,4,4-trimethylpentene to CD rats for 40 to 46 days (during premating, mating, gestation and up to lactation day 4) at dosages of up to 1 000 mg/kg bw/d did not reveal any indications for an impairment of reproductive performance and capability or peri/postnatal viability and performance of offspring up to postnatal day 4 at a screening level. Thus NOAEL of 1 000 mg/kg bw/d indicates that reproductive toxicity is no relevant endpoint for 2,4,4-trimethylpentene.

4.1.3.2 Workers

4.1.3.2.1 General aspects of occupational risk assessment

2,4,4-Trimethylpentene is a liquid with a vapour pressure of 5 790 Pa at 25°C. It is soluble in organic solvents and to a lesser extent in water. 2,4,4-Trimethylpentene is primarily used as chemical intermediate for the production of aldehydes, alkyl phenols and polymers. A small portion is used in the metal industry as stabilizer for degreasing agents. Handling of 2,4,4-trimethylpentene during production and further processing is the main source for occupational exposure. There is one relevant exposure scenario at the workplace which is described and discussed in section 4.1.1.2. Exposure routes to be considered are inhalation against 2,4,4-trimethylpentene vapours and skin contact with the liquid substance. For worker risk assessment the shift average values as reported in table 4.1.1.2.2.A and table 4.1.1.2.2.B are taken forward to risk characterisation.

The toxicological data of 2,4,4-trimethylpentene 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 to 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. The toxicity profile of 2,4,4-trimethylpentene does not appear to be very marked, repeated dose toxicity reflecting the most sensitive endpoint.

body weight, rat	250 g
body weight, human	70 kg
respiratory rate, rat (reduced activity)	0.8 l/min/kg
respiratory volume worker during 8 h (moderate activity)	10 m^3

Systemic availability for different routes of exposure.

For the majority of toxicological endpoints 2,4,4-trimethylpentene 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.

There are no data from toxicokinetics to decide on absorption of 2,4,4-trimethylpentene via the dermal, oral and inhalation route. From the physico-chemical data of 2,4,4-trimethylpentene it is derived that the substance shows probably a good bioavailability after oral, dermal and inhalation exposure. It is proposed to take forward for the risk characterisation a value of 100 % for all routes. Concerning dermal absorption, uncertainties might arise from evaporation. From physicochemical data it is calculated as a rough estimate that an amount of 1 mg 2,4,4-trimethylpentene per cm^2 applied might be completely evaporated within approximately 12-19 seconds (30-20°C at the surface of the hand) non-occlusive exposure conditions provided. (see chap. 4.1.1.2.1) This might essentially limit the duration of skin contact thereby mitigating absorption.

For risk assessment at the workplace in a first step 100 % dermal absorption is assumed as worst case. Since no scenario with concern is identified on that background there is no need for a more detailed evaluation of dermal absorption.

Occupational exposure and internal body burden

In order to assess risks by combined exposure a so-called “internal body burden” is identified. This parameter combines the contribution of dermal and inhalation exposure of workers to a total effective dose.

In table 4.1.3.2.A the route specific exposure values are listed and the internal body burden of workers as result of combined exposure via inhalation and dermal exposure is identified. From this calculation skin contact appears to be the major source for the internal body burden of 2,4,4-trimethylpentene. It has to be kept in mind, however, that dermal absorption might be overestimated because of evaporation.

Table 4.1.3.2.A: 2,4,4-Trimethylpentene exposure levels which are relevant for occupational risk assessment and internal body burden

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 and further processing	5	210[(3)]	50	210	260

[(1)] systemic availability after inhalation: default value 100%; internal body burden: shift average x 10 m^3
[(2)]systemic availability after dermal contact: default value 100%
[(3)]use of unsuitable gloves

Calculation of MOS and MOE 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. If the route of application in animal studies for a certain endpoint is different from the actual occupational exposure, the dose units of the experimental data are adapted to the exposure situation previously to MOS calculation. In case of 2,4,4-trimethylpentene, oral doses from experimental studies have to be converted to air concentrations or dermal exposure levels. For this procedure the physiological default values from above are used to modify the dose unit of effects data. As result a so-called "starting point" for risk assessment is identified.

MOS values for inhalation and dermal route are considered separately. The combined MOS-value is calculated as quotient of the internal NAEL (i.e. the external NOAEL multiplied by the percentage of absorption) and total internal body burden. In case of acute toxicity, different data for different application routes are available for 2,4,4-trimethylpentene. For the assessment of combined risks the oral study is used, giving the lowest NOAEL reported.

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 2,4,4-trimethylpentene.

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 2,4,4-trimethylpentene, 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.

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 for each endpoint an exposure level is identified for 2,4,4-trimethylpentene which by direct comparison with the occupational exposure level 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 if exposure exceeds 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 the occupational 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 breath 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 corresponding 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 a default intraspecies variation factor for local effects is not defined.

Duration adjustment

From substance-specific data for various chemicals it is known, that the duration of a study may significantly influence the NOAEL. Longer study duration frequently implies a lower NOAEL. Based on average values, duration adjustment for systemic effects for subacute to chronic exposures uses the default factor of 6 (Kalberlah, F. & Schneider, K., 1998). Data from studies with 2,4,4-trimethylpentene (28-day oral study in rats from Huntigton Life Sciences, 1997a and a 6-weeks oral study in rats from OECD TG 421) support this factor. Since there is no better information available this value will be used for duration adjustment of repeated dose toxicity data on 2,4,4-trimethylpentene concerning systemic effects.

Where adaptation of daily doses is necessary, e.g. in the calculation of totally administered amounts of 2,4,4-trimethylpentene, 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 therefore is supposed to result in a central tendency point 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, be 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 Occupational risk assessment

Acute Toxicity

Acute toxicity by inhalation

From an acute inhalation test with twenty female Wistar rats per dose for 4 hours a LC50 value of 30 000 mg/m^3 is reported. Since no further information is available the LC50 is chosen as starting point for MOS calculation.

Evaluation of the MOS values has to account for the following aspects: (i) study duration was 4 hours compared to occupational exposure of 8 hours, (ii) physiological differences between humans at rest and workers account for a factor of 1.5, (iii) for adaptation of the LC50 to an air concentration without acute toxicity a default factor of 3 is proposed, (vi) human intraspecies variation is accounted for by a factor of 5, (v) an uncertainty factor of 3 seems appropriate because the information used for the assessment is of low quality. Altogether the

minimal MOS calculates to 135 (8/4 x 1.5 x 3 x 5 x 3). The critical exposure level is identified as 222 mg/m^3 (30 000 mg/m^3 / 135).

The shift average value for inhalation is reported as 5 mg/m^3. The resultant MOS value calculates to 6 000 (30 000 / 5, compare table 4.1.3.2.B), which is well above the minimal MOS, thus not leading to concern.

conclusion (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

Acute toxicity by dermal and combined contact

In a rat limit test with occlusive exposure for 24 hours a dose of 2 000 mg/kg did not result in apparent signs of systemic toxicity. As starting point for MOS calculation the human dose corresponding to the dermal NOAEL in rats is calculated to 140 000 mg/person (2 000 mg/kg x 70 kg).

Evaluation of the MOS values has to account for the following aspects: (i) metabolic rate scaling from rats to humans reveals a factor of 4, (ii) human intraspecies variation is accounted for by a factor of 5, (iii) an uncertainty factor of 3 is proposed in analogy to a standard estimate, although it might assumed, on the background of the dermal NOAEL, that this factor errs on the side of caution. Altogether the minimal MOS calculates to 60 (4 x 5 x 3). The critical exposure level is identified as 2 300 mg/person (140 000 mg/person / 60).

The combined and dermal exposure level for production and further processing are reported to 215 resp. 210 mg/p/d (compare table 4.1.3.2.B). The corresponding MOS values calculate to 650 (140 000 / 215) resp. 666 (140 000 / 210) which give no reason for concern.

conclusion (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

Table 4.1.3.2.B: Acute toxicity, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	30 000 mg/m^3			140 000 mg/p			140 000 mg/p		
Minimal MOS	135			60			60		
Critical exposure level	222 mg/m^3			2 300 mg/p/d			2 300 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/p)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
Production and further processing	5	6 000	ii	210	666	ii	260	650	ii

Irritation/Corrosivity

Dermal and eye irritation

In studies with rabbits mild skin irritation after 4-hours semi-occluded application and slight eye irritation was observed for 2,4,4-trimethylpentene. After prolonged contact blanched white skin, eschar formation and exfoliation point to defatting as a hazardous effect. Additionally there are indications for a irritation potential from structurally similar substances (see chap. 4.1.2.4) which are reported to cause skin irritation. Therefore 2,4,4-trimethylpentene is proposed to be classified as a skin irritant.

According to the exposure assessment skin contact with 2,4,4-trimethylpentene critically depends on the proper use of suitable gloves. Even though the use of PPE generally is highly accepted in the large scale chemical industry there is a possibility that unsuitable glove material is provided. Therefore the actual extent of protection cannot properly be estimated. However, there is no indication for prolonged contact in the range of hours. On the grounds that control measures exist for 2,4,4-trimethylpentene which should be able to efficiently minimize exposure conclusion ii is proposed. However, these control measures must be implemented and complied with to reduce the risk of skin damage.

conclusion (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

Acute respiratory irritation

No animal studies are available concerning the irritation potential of 2,4,4-trimethylpentene after inhalation. Three human volunteers were exposed for 5 minutes to a mixture of 8-Olefines (ca. 75% 2.4.4-Trimethylpentene-1 and ca. 15% 2.4.4-Trimethylpentene-2). A concentration of 465 mg/m^3 resulted in irritating effects on the mucosa of nose and throat. 279 mg/m^3 smelled distinctly but did not cause irritating effects.

The highest inhalation exposure level at the workplace is a short-term value up to 90 mg/m^3. This value is below the irritation level, although it is recognized that the duration of occupational exposure for this short term level can last up to 45 minutes. Taking into account that during all the years of use no notice of specific case reports has been given, a damage of the airways by acute irritation of TMP at the workplace is not anticipated.

Direct aspiration of liquid 2,4,4-trimethylpentene into the airways might cause a significant hazard, however this kind of exposure is not probable at the workplace. There is no reason for concern.

conclusion (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

Sensitisation

Skin sensitisation

From animal data no skin sensitising properties of 2,4,4-trimethylpentene can be derived. There is no concern with respect to skin sensitisation at the workplace.

conclusion (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

Respiratory sensitisation

No information on the sensitising potential of the substance at the respiratory tract is available. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. However, 2,4,4-trimethylpentene is not expected 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) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Repeated dose toxicity

Local effects by inhalation

Extrapolation from the irritating concentration of 465 mg/m³ (5 minutes, volunteers, see chapter 4.1.2.4) to an irritation level for repeated exposure is considered too speculative. In the "german list of occupational exposure levels" (TRGS 900) the OELs for 32 substances which are solely classified as irritants spread over a range from 5 mg/m^3 until 1 200 mg/m^3. The central 50% of the OELs lie between 70 and 450 mg/m^3. Looking at this distribution of OELs for irritating substances there seems to be no concern for local effects by repeated inhalation.

conclusion (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

Local effects by dermal contact

2,4,4-Trimethylpentene has irritating properties and is proposed to be classified as a skin irritant (see chapter 4.1.2.4). No further information about local effects after repeated dermal contact is available.

According to the exposure assessment skin contact with 2,4,4-trimethylpentene critically depends on the proper use of suitable gloves. Even though the use of PPE generally is highly accepted in the large scale chemical industry there is no information on the suitability of used gloves. Therefore the actual extent of protection cannot properly be estimated. However, daily dermal contact seems to be short. On the grounds that control measures exist for 2,4,4-trimethylpentene which should be able to minimize exposure conclusion ii is proposed.

However, these control measures must be implemented and complied with to reduce the risk of skin damage.

conclusion (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

Systemic effects by inhalation, dermal contact and combined exposure

Inhalation data concerning this endpoint are not available. From a valid 28-day oral study in rats a NOAEL of 300 mg/kg/day is reported for female animals. At higher doses (1 000 mg/kg/day) adaptive liver effects were observed (Huntigton Life Sciences 1997a). In another oral reproductive developmental screening test basophilic cortical tubules were observed in all treated male rats (Huntington Life Sciences 1997b). The described nephrotoxic effects are associated with α_{2u}–globulin accumulation. On that background these effects are considered to be of minor importance for humans (for further discussions see chapter 4.1.2.6). The NOAEL of 300 mg/kg/day, derived from the 28-day oral study is taken for the following calculations.

For systemic effects by inhalation, the NOAEL of 300 mg/kg/day is converted to an internal human dose of 21 000 mg/person/day (300 mg/kg/day x 70 kg). For this calculation 100% oral absorption is assumed. Against the background of a 100% absorption by inhalation the corresponding airborne concentration is calculated to be 2 100 mg/m^3 (21 000 mg/person/day / 10 m^3).

In evaluation of MOS values the following aspects have to be considered: (a) metabolic rate scaling from rats to humans yields a factor of 4, (b) duration adjustment from a subacute to a chronic study reveals a factor of 6, (c) human intraspecies variation is accounted for by a factor of 5. No additional uncertainty factor is applied, because the effects observed at high doses are considered to be an adaptive response with a low adverse character. Altogether the minimal MOS for systemic effects after repeated exposure calculates to 120 (4 x 6 x 5). The corresponding critical exposure level, is 17.5 mg/m^3 2 100 mg/m^3 /120) .

For the only occupational scenario (production and further processing) the distance between the inhalative exposure of 5 mg/m^3 and the critical exposure level of 17.5 mg/m^3 seems big enough to draw for conclusion ii.

Both for the dermal and oral routes of exposure a 100% absorption is assumed. For the dermal and combined exposure situation (which is predominated by skin contact) the critical exposure level of 175 mg/person/day reaches borderline (see table 4.1.2.3.C). It has to be kept in mind that dermal exposure probably is lower than estimated because of significant evaporation (see explanations to the systemic availability for different routes of exposure in chapter 4.1.3.2.1) and because of some protection by gloves (see chap. 4.1.1.2.1). For these reasons no concern will be expressed.

conclusion (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

Table 4.1.3.2.C Repeated dose toxicity, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	2 100 mg/m^3			21 000 mg/p/d			21 000 mg/p/d		
Minimal MOS	120			120			120		
Critical exposure level	17.5 mg/m^3			175 mg/p/d			175 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
Production and further processing	5	420	ii	210[(1)]	100	ii	260[(1)]	98	ii

[(1)]On account of the high vapour pressure of TMP, the resulting retention time of the substance on the gloves or the skin is shortened and lower levels of dermal exposure than the estimated ones are to be expected.

Mutagenicity

From relevant in vitro studies there is no evidence for mutagenicity of 2,4,4-trimethylpentene. There is no reason for concern.

conclusion (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

Carcinogenicity

No cancer studies on 2,4,4-trimethylpentene are available. From the available data there is no indication for concern with respect to 2,4,4-trimethylpentene. (for further discussion see chap. 4.1.2.8)

conclusion (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

Reproductive toxicity, fertility and developmental effects

Fertility and developmental effects by inhalation, dermal and combined exposure

In an OECD screening test (OECD 421) in rats dosages up to 1 000 mg/kg/day did not reveal indication for reproductive toxicity. There is no concern with respect to this endpoint for workers.

conclusion (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

4.1.3.2.3 Summary of occupational risk assessment

Table 4.1.2.3.D summarises the conclusions of the occupational risk assessment for 2,4,4-trimethylpentene. For all toxicological endpoints conclusion ii is expressed.

Table 4.1.3.2 D Endpoint-specific overall conclusions

Toxicological endpoints		Overall conclusion
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Acute respiratory tract irritation		ii
Dermal and Eye irritation/ corrosivity		ii
Skin and respiratory sensitization		ii
Local effects by repeated exposure	inhalation	ii
	dermal	ii
Systemic effects by repeated exposure	inhalation	ii
	dermal	ii
	combined	ii
Mutagenicity		ii
Carcinogenicity		ii
Reproductive toxicity (fertility impairment, developmental toxicity)		ii

Risk estimation is mainly based on oral studies. Since workers are exposed either by inhalation or by skin contact, a route to route transformation is necessary. In the risk assessment a for inhalation, dermal and oral absorption a default value of 100% is assumed. On the background of the exposure assessment and the proposed critical exposure levels, endpoint-specific health risks seem to be comparably low, leading to no concern for the only scenario 1 (production and further processing).

4.1.3.3 Consumers

Since a consumer exposure does not exist, a health risk of consumers regarding Acute toxicity, Irritation, Corrosivity, Sensitization, Repeated dose toxicity, Mutagenicity, Carcinogenicity, and Reproductive toxicity is not expected.

Conclusion (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.

4.1.3.4 Man exposed indirectly via the environment

The estimations for human exposure to 2,4,4-trimethylpentene via the environment are summarised in section 4.1.1.4 for the local and regional scenario. The total daily dose for the

local scenario, which is mainly due to intake of roots and fish, amounts to 1.11 mg/kg bw/d. For the regional scenario, inhalation via air is the main route of intake. However, with a total daily dose of only 0.97 ng/kg bw/d, this exposure is negligible and will be neglected for the risk characterisation.

When considering possible risks to human health arising from indirect exposure to 2,4,4-trimethylpentene via the environment the key areas of concerns are for repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity.

Repeated dose toxicity

From a valid 28-day oral study in rats a NOAEL of 300 mg/kg/day is reported. At higher doses (1 000 mg/kg/day) adaptive liver effects were observed. In another oral study (a reproductive developmental screening test), nephrotoxic effects were observed in male Sprague-Dawley CD-1 rats at ≥100 mg/kg bw/day (LOAEL), mainly in the cells of the proximal tubuli. These renal lesions were induced by a dose-related increased accumulation of α_{2u}-Globulin, a sex- and species-specific phenomenon. On that background these effects are considered to be of minor importance for humans (for further discussions see chapter 4.1.2.6).

The NOAEL of 300 mg/kg/day, derived from the 28-day oral study with rats, is used as starting point for the risk characterisation for repeated dose toxicity.

For a conclusion about the appropriateness of the MOS for this endpoint, the following aspects were considered and taken into account:

- *Overall confidence in the database*

The data taken into account for performing the risk characterisation have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognised guidelines. The judgement can be based on the database and there are no reasons to assume limited confidence.

- *Uncertainty arising from the variability in the experimental data*

There are no reasons to assume a special extent of uncertainty which have to be taken into account.

- *Intra- and interspecies variation*

Data on kinetics of the substance that might give an indication of the intraspecies and interspecies variability are not available. Data on the toxicodynamics are only available for one species (rat). There are no reasons to assume a special extent of uncertainty which have to be taken into account.

- *Dose response relationship*

There is no reason to assume a special concern.

- *Nature and severity of the effect*

2,4,4-Trimethylpenten orally administered to rats for 28 days induced changes in the liver and the kidneys at relatively high doses. These effects are considered to be an adaptive response with a low adverse character. There is no reason to assume a special concern.

- *Differences in exposure (route, duration, frequency and pattern)*

The estimated oral intake via food and water is compared with an oral NOAEL derived from 28 day toxicity studies in rats. This procedure is consistent with established risk assessment methodology.

- *The human population to which the quantitative and/or qualitative information on exposure applies*

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- *Other factors*

There are no other factors known that might require a particular margin of safety.

MOS for the local exposure scenario

The daily intake was calculated to be 0.46 mg/kg bw/d. The margin of safety between the

exposure level of	1.11 mg/kg bw/d

and the

oral NOAEL of	300 mg/kg bw/d

is judged to be sufficient.

Conclusion (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.

Mutagenicity

On the basis of negative results from bacterial mutation tests and a chromosomal aberration test with human lymphocytes in vitro there is no evidence of a genotoxic potential of 2,4,4-trimethylpentene.

Conclusion (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.

Carcinogenicity

There are no experimental data on carcinogenicity of 2,4,4-trimethylpentene available. Studies with the structurally analogous substance 2,4,4-trimethylpentane have shown that the formation of renal tubule tumours in male rats is linked through α_{2u}-Globulin nephropathy. However, this toxicity only occurs in male rats of strains that synthesize α_{2u}-Globulin and not in other species, including humans. In a reproductive developmental screening test with 2,4,4-trimethylpentene dose-related increased incidences of kidney lesions were observed in male, but not in female rats. Results of α_{2u}-Globulin immunohistochemistry in rat kidneys from this study have shown that 2,4,4-trimehtylpentene induces α_{2u}-Globulin nephropathy. Based on the results of this study and the prior research on α_{2u}-Globulin nephropathy, the kidney effects associated with 2,4,4-trimethylpentene-induced α_{2u}-Globulin nephropathy can be considered without relevance to carcinogenicity risk assessment. Taking into account the

negative mutagenicity data it is concluded that carcinogenicity should not be an endpoint of concern for humans.

Conclusion (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.

Toxicity for reproduction

Oral administration of 2,4,4-trimethylpentene to CD rats for 40 to 46 days (during premating, mating, gestation and up to lactation day 4) at dosages of up to 1 000 mg/kg bw/d did not reveal any indications for an impairment of reproductive performance and capability or peri/postnatal viability and performance of offspring up to postnatal day 4 at a screening level. Thus NOAEL of 1 000 mg/kg bw/d indicates that reproductive toxicity is no relevant endpoint for 2,4,4-trimethylpentene.

Conclusion (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.

4.1.3.5 (Combined exposure)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

see chapter 4.1.1.1

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

2,4,4-Trimethylpentene is not explosive.

4.2.2.2 Flammability

2,4,4-Trimethylpentene is highly flammable.

4.2.2.3 Oxidising potential

Testing for this property is not applicable due to the physical nature of the substance.

4.2.3 Risk characterisation

4.2.3.1 Workers

2,4,4-Trimethylpentene is highly flammable. Adequate worker protection measures must be observed. Risk reduction measures beyond those which are being applied already are not considered necessary.

Conclusion (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.

4.2.3.2 Consumers

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

Environment

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

Further exposure information is needed with respect to the releases into the waste water treatment plants and into surface water for both production sites and for 23 out of 25 processing sites. Site specific exposure information is needed also for 2 sites for refinement of the assessment of non compartment specific effects relevant for the food chain. (probably covered by previous information requests). In addition an activated sludge respiration inhibition test (OECD 209) and prolonged daphnia magna reproduction test (OECD 211) are needed before definitive conclusions can be drawn. Test and exposure information requirements have been agreed at TC NES IV '07.

Conclusion (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.

There is no concern with respect to the releases into the waste water treatment plants, surface waters and sediment for processing sites A and B and non compartment specific effects relevant for the food chain for one production site and 24 out of 25 processing sites. There is no concern for the atmospheric and terrestrilal compartment from the production and use of the substance. 2,4,4-trimethylpentene does not meet the PBT criteria.

Human Health

Workers

Conclusion (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.

The toxicity profile of 2,4,4-trimethylpentene does not appear very marked. In combination with the dermal and inhalation exposure levels at the workplace no occupational scenarios of concern have been identified.

Consumers

Conclusion (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.

Man exposed indirectly via the environment

Conclusion (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.

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APPENDIX A1

Calculation of Evaporation Time

In the case of 2,4,4-trimethylpentene, the predominant effect reducing potential dermal exposure is the very high volatility of the substance (vapour pressure 5.8 kPa) which leads to considerable low retention times of the substance on the skin or on the protective gloves. This exposure reducing effect cannot be considered if workers have continuous direct contact with the substance, e.g. dipping hands into the substance. For the area of production and further processing of 2,4,4-trimethylpentene, this situation is regarded to be rather non-probable. Furthermore, it is assumed, that non-occlusive exposure is the predominant exposure situation.

For the purpose of determining the evaporation rate of 2,4,4-trimethylpentene, an equation was used which was derived within the framework of a research project (Weidlich, Gmehling 1986, Gmehling et al., 1989). This project was aimed at calculating airborne concentrations of substances when emitted from liquid mixtures under consideration of the evaporation and the spreading of the substance at the workplace. For calculating the evaporation times of substances, an equation was derived based on the mass transfer at the interface between the liquid and the vapour (two-film-theory). Mass transfer during evaporation occurs until the equilibrium state is achieved. The main influence on evaporation is the transfer through the interface.

For pure substances, the following equation is used:

$$t_{(s)} = \frac{m \cdot R \cdot T}{M \cdot \beta \cdot p \cdot A} \cdot K$$

t: time [s]
m: mass, EASE estimate, [mg] (per cm²)
R: gas constant: 8.314 J K^{-1} mol^{-1}
T: skin temperature [K]
M: molar mass [g mol^{-1}]
β: coefficient of mass transfer in the vapour phase [m h^{-1}], for calculation: β = 8.7 m/h, see below
p: vapour pressure of the pure substance [Pa]
A: area, EASE: 1 cm^2
K: conversion factor

The skin temperature amounts normally to 28 – 32°C (ambient temperature: 20 – 22°C). The reduction of the skin temperature and accordingly of the vapour pressure caused by the evaporation process is not considered in the equation. This might be done by choosing a lower mean temperature for the evaporation process.

The coefficient of mass transfer β is described based on empirical studies:

$\beta = (0.0111 * v0.96 * Dg0.19) / (\nu 0.15 * X0.04)$

Dg : coefficient of diffusion, gas phase
v: velocity of air [m/h]
ν: kinematic viscosity of air [m²/h]
X: length of the area of evaporation in the direction of the air stream [m]

In the above given equation, the main influencing parameter the velocity of the air (v). At workplaces v is often between 0.3 m/sand 0.6 m/s (a velocity higher than 0.5 m/s is felt as non-convenient). Since the hands from which a substance evaporates are often in motion, the air velocity might be higher. For a conservative approach, the lower value (0.3 m/s) was chosen.

For different organic solvents, D_g is approx. 0.05 m^2/h. As a range might serve 0.03 – 0.06 m²/h, so that $Dg^{0.19}$ ranges between 0.58 and 0.51.

A literature value was taken for the kinematic viscosity of air (5.4396 10^{-2} m²/h).

The parameter X, representing the length of the area of evaporation in the direction of the air stream [m] is because of its low exponent (0.04) not very influencing. For the calculation, a length of 10 cm was taken.

Taking into account a rather low velocity of air (0.3 m/s), β is about 8.7 m/h. This value is in good correspondence with experimental values of similar substances: For ethyl acetate β amounts to 8 m/h (air velocity 0.31 m/s) and for butyl acetate, a value of 9.2 m/h (air velocity 0.31) was obtained.

For 2,4,4-trimethylpentene and the EASE estimate of 1 mg/cm², an evaporation time of 12 seconds (T = 30°C) is calculated. For 2,4,4-trimethylpentene on the gloves, an assumed temperature of 20°C leads to a evaporation time of 19 seconds. These values should be regarded to represent the order of magnitude, since it is not known in how far the interaction of the skin with the substance influences the evaporation time. The error caused by this interaction is regarded to be higher than the one caused by the uncertainty of the calculation of β. For different substances (7 substances were investigated) β differs about ± 15 %.

[1] U. Weidlich, J. Gmehling: Expositionsabschätzung. Eine Methode mit Hinweisen für die praktische Anwendung, Schriftenreihe der Bundeanstalt für Arbeitsschutz und Arbeitsmedizin – Forschung – Fb 488, Verlag NW, Bremerhaven (1986)

[2] J. Gmehling, U. Weidlich, E. Lehmann. N. Fröhlich: Verfahren zur Berechnung von Luftkonzentrationen bei Freisetzung von Stoffen aus flüssigen Produktgemischen, Teil 1 und 2, Staub – Reinhaltung der Luft, 49, p. 227 – 230, 295 – 299 (1989)

The report provides the comprehensive risk assessment of the substance 2,4,4-trimethylpentene. 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. 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

There is no concern identified for the atmosphere and the terrestrial environment. Further information is needed before conclusions can be drawn regarding the aquatic compartment and non compartment specific effects via the food chain. Further information is also needed regarding the effects on the functioning of waste water treatment plants. Since it is no longer possible to submit this information under the Existing Substances Regulation it is proposed to consider the substance further under the REACH regulation.

For human health, there is no concern for any of the considered populations.