FOREWORD INTRODUCTION

3,5,5-TRIMETHYLCYCLOHEX-2-ENONE (ISOPHORONE)

CAS N°: 78-59-1

SIDS Initial Assessment Report

For

SIAM 16

Paris; France, 27– 30 May 2003

1.	**Chemical Name:**	3,5,5-Trimethylcyclohex-2-enone (Isophorone)
2.	**CAS Number:**	78-59-1
3.	**Sponsor Country:**	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn-Bad Godesberg
4.	**Shared Partnership with:**	
5.	**Roles/Responsibilities of the Partners:**	
•	Name of industry sponsor /consortium	Atofina SA: Dr. J. Bakès /Dr. J.- F. Régnier (France); Daicel Chemical Industries, Ltd. Dr. T. Baba (Japan); Degussa AG: Dr. R. Ebert/ Dr. M. Weiß (Germany); Dow Chemical: Dr. T. Cawley (U.S.);
•	Process used	See next page
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7.	**Review Process Prior to the SIAM:**	last literature search (update): December 2002 (Human Health + Ecotoxicology): databases BIODEG, BIOLOG, DATALOG, ECDIN, ECOTOX, TOXLINE and Web of Science
8.	**Quality check process:**	As basis for the SIDS-Dossier the IUCLID was used. Data have been checked and validated by an external peer reviewer (FhG, Hannover). A final evaluation of the human health part in the documents has been performed by the Federal Institute for Risk Assessment (BfR), Berlin. A final evaluation of the ecotoxicological part in the documents has been performed by the Federal Environment Agency (UBA), Berlin.

9. Date of Submission: 21. February 2002

10. Date of last Update:

11. Comments: **OECD/ICCA - The Peer Review Process**

A quality control on the full SIDS dossier submitted by industry was performed by an independent peer reviewer (FhG, Hannover) with experience in toxicological and ecotoxicological assessment. This quality control process includes:

a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET

Review of data and assessment of the quality of data

Review of data evaluation

Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications

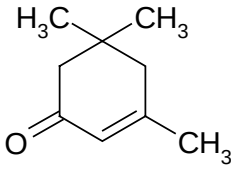
Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications
(if original reports are missing: reliability (4), i.e. reliability not assignable)

Review of validity of structure-activity relationships

Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)

In case of data gaps, review of testing plan or rationale for not testing

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	78-59-1
Chemical Name	3,5,5-trimethylcyclohex-2-enone (Isophorone)
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Upon oral and inhalative administration, isophorone is well absorbed and rapidly distributed through the body of rats and rabbits. While part of the absorbed isophorone is excreted unchanged via urine and exhaled air, metabolites are mainly excreted as glucuronides. The tendency of isophorone to bioaccumulate is very low, since within 24 hours after administration more than 93% of orally administred isophorone was excreted by rats.

The acute toxicity in laboratory animals is low to moderate (oral LD50 $\geq$ 1500 mg/kg bw; dermal LD50 $\geq$ 1200 mg/kg bw; inhalative LC50 = 7000 mg/m^3). Isophorone is an eye irritant and a respiratory irritant but does not irritate the skin. It is not sensitizing in animal studies.

In subchronic studies, oral administration of high doses of isophorone (NOAEL (male rat, 90 days) = 102.5 mg/kg bw/day, NOAEL (female rat, 13 weeks) = 500 mg/kg bw/day; NOAEL (male mouse, 16 days) = 500 mg/kg bw/day, NOAEL (female mouse, 16 days) = 125 mg/kg bw/day; NOAEL (dog, 90 days) $\geq$ 150 mg/kg bw/day) caused no significant toxic effects (all NOAELs are based on slight (< 14%) reductions in body weight gain). After inhalational administration nose and eye irritation and blood and liver changes were observed (NOAEL (rat, 28 days) < 208 mg/m^3).

Although in one mouse lymphoma assay a positive result was observed, the majority of *in vitro* genotoxicity studies revealed clearly negative results. Together with the negative *in vivo* results and the negative DNA binding assay, the overall conclusion is that isophorone is not mutagenic.

There was some evidence of carcinogenicity of isophorone in male rats (kidney tumors, preputial gland carcinomas). The kidney tumors can be attributed to an α2u-globulin associated mechanism. The observed nephropathy in male rats is therefore irrelevant to other species. As the preputium is only investigated histopathologically when gross lesions are found, neither true tumor incidences from this study nor from historical controls are available. Therefore, the higher incidence of preputial gland tumors in high dose male rats cannot be put into perspective. There was equivocal evidence of carcinogenicity for male mice (liver tumors, mesenchymal tumors of the integumentary system). There was no evidence of carcinogenicity of isophorone in female rats and mice.

There is no evidence indicating that isophorone interferes adversely with the reproduction. No changes were observed in pregnancy rates, litter sizes, pups abnormalities or in histopathological examinations of the reproduction organs after long-term studies. In an inhalation teratogenicity study, the NOAEL for maternal toxicity was 289 mg/m³ (based on a reduction in body weight gain of less than 7%).

Isophorone was neither embryotoxic nor teratogenic up to the highest test concentration of 664 mg/m³ isophorone.

Environment

Isophorone has a melting point of –8.1 °C, a solubility in water of 14.5 g/l at 20 °C, and a vapour pressure of 40 Pa at 20 °C. The measured log Kow is 1.67.

According to a Mackay Level I model calculation the main target compartments for isophorone will be the

hydrosphere (87.6 %) and atmosphere (11.7 %). The calculated Henrys' law constant of 0.38 $Pa.m^3/mol$ indicates evaporation from surface waters within several days. With a calculated Koc of 77 l/kg the sorption potential to soil or sediment organic matter is expected to be low.

In the atmosphere, isophorone is rapidly removed by reaction with ozone with an estimated half-life of 23 minutes. The calculated half-life for photodegradation by reaction with OH radicals is 16 h. Isophorone can be considered to be readily biodegradable. In surface waters, the main removal mechanisms are expected to be biodegradation and volatilization. Photolytical degradation in surface waters is expected to be of minor importance. Furthermore, hydrolytic degradation is not to be expected. Experimentally determined BCF values below 10 l/kg indicate a low bioaccumulation potential.

The lowest valid acute test results of aquatic testing determined for fish, daphnia, algae and bacteria were as following:

Cyprinodon variegatus:	96h-LC50 = 140 mg/l
Daphnia magna:	48h-EC50 = 120 mg/l
Scenedesmus subspicatus:	72h-EbC50 = 475 mg/l; 72h-EbC10 = 64 mg/l
Activated sludge:	3h-EC50 = 100 mg/l

In 3 fish early-life-stage tests with *Pimephales promelas* NOEC-values of 4.2 mg/l (32 d), 15.6 mg/l (32 d) and 11 mg/l (35 d) were obtained for the endpoint growth (measured as weight). The geometric mean of these 3 NOEC is 8.9 mg/l. Based on this value, a PNEC of 0.178 mg/l is calculated using an assessment factor of 50.

For the terrestrial compartment, a PNEC could not be calculated. Isophorone spiked into soils is expected to be rapidly removed by biodegradation, thus the test substance concentration will be unstable during the test period.

Exposure

The production volume of Isophorone is approx. 100,000 t/y world wide. Isophorone is widely used as a solvent for a number of synthetic resins and polymers, as well as in special application paints and printing inks. It is a chemical intermediate and an important solvent in certain herbicide formulations.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical is currently of low priority for further work based on its low hazard potential. The substance is an eye irritant. Although this does not warrant further work, this property should nevertheless be noted by chemical safety professionals and users.

SIDS Initial Assessment Report

1 IDENTITY

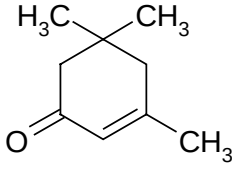
1.1 Identification of the Substance

CAS Number: 78-59-1

IUPAC Name: 3,5,5-Trimethylcyclohex-2-enone

Molecular Formula: $C_9H_{14}O$

Structural Formula:



Molecular Weight: 138.21 g/mol

Synonyms: Isophorone
α-Isophorone
Isoacetophorone
2-cyclohexen-1-one, 3,5,5-trimethyl-
3,5,5-Trimethyl-2-cyclohexene-1-one
3,5,5-Trimethyl-2-cyclohexen-1-one
3,5,5-Trimethyl-2-cyclohexenone
1,1,3-Trimethyl-3-cyclohexene-5-one
Isooctaphenone

1.2 Purity/Impurities/Additives

Substance type: organic

Purity: 97.5 - 99.6 %

Impurities: ca. 1.5 % 3,5,5-trimethylcyclohex-3-en-1-one (471-01-2)

ca. 0.15 % xylitol (87-99-0)

ca. 0.12 % 2,6-dimethylhepta-2,5-dien-4-one (504-20-1)

<= 0.1 % water

ca. 0.06 % trimethylcyclohexanone (Cas No. unknown)

ca. 0.05 % 3,3,5,5-tetramethylcyclohexanone (14376-79-5)

ca. 0.01 % 2,3,5,5-tetramethylcyclohexanone (Cas No. unknown)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	liquid	
Melting point	-8.1 °C	Braithwaite, 1995
Boiling point	215.3 °C	Braithwaite, 1995
Relative density	0.9215 g/cm³	Auergesellschaft, 1998
Vapour pressure	0.4 hPa at 20 °C (measured)	Huels AG, 1981
Water solubility	14.5 g/l at 20 °C	Veith et al., 1980
Partition coefficient n-octanol/water (log value)	1.67 (measured)	Veith et al., 1980

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The worldwide production of isophorone is estimated to be in the order of 100,000 tons per year. Production sites are in the EU (Germany, France), the U.S., Japan and India. Isophorone is widely used as a solvent for a number of synthetic resins and polymers, as well as in special application paints and printing inks. It is a chemical intermediate and an important solvent in certain herbicide formulations.

In the Danish Product Register (Feb. 2002), a total of 38 isophorone containing products with a total quantity of 30 t/a were listed, all of which were of industrial use. Product types are pesticides, reprographic agents and paints, lacquers and varnishes. In the Swedish Product Register (Feb. 2002), 33 products containing isophorone are listed, none of those are consumer products. “Paints for metal products” is given as the main use.

In the Swiss Product Register (Dec. 2001, a total of 152 products containing isophorone are listed, 53 of which are consumer products. The majority of the consumer products containing isophorone are selective herbicides (36/53), while professional use is focussed on lacquers and varnishes (76/99) and solvents (12/99).

In the Norwegian Product Register (2003) 11 products containing a total quantity of 3 t isophorone were registered in 2001. The most frequent reported use category is paints.

In the Finnish Product Register (2003) 25 products containing a total amount of 67.5 t isophorone were registered in 2001. The most frequent use categories reported are binding agents, surface treatment, colouring agents and pesticides.

2.2 Environmental Exposure and Fate

2.2.1 Environmental Exposure

Releases into the environment may occur during production, processing and direct use of isophorone as a solvent. The following exposure information for the single production sites is available:

Germany:

In the German production plant, offgas is quantitatively combusted in a thermal oxidizer. Process wastewater of approx. 16,000 m³/year contains << 1 mg/l isophorone (i.e. total amount of << 16 kg isophorone/year) is treated in a wastewater treatment plant. Higher contaminated process wastewater is concentrated and combusted in a thermal oxidizer (Degussa, 2002).

France:

The concentration of isophorone in the receiving river (Arc) is estimated to be well below 1 ppm.

U.S.:

At the U.S. production site, emissions to air were 468 kg (1999), 310 kg (2000), and 1,221 kg (2001). Emissions to waste water were 1,080 kg (1999), 1,027 kg (2000), and 792 kg (2001). Effluent from the waste water treatment plant going into Kanawha River contained approx. 42 ppb isophorone, which amounts to a total of approx. 207 kg/year (The Dow Chemical Company, 2002).

Japan:

Approx. 6,300 m³ of process wastewater/year containing 12 g/l isophorone (80 t/year) are subjected to activated sludge treatment and drained thereafter (Daicel, 2000; further information not available).

For the life-cycle-steps processing and direct use no exposure information characterizing the present situation is available. From the use as a solvent in herbicides, an exposure of the terrestrial compartment is to be expected.

2.2.2 Environmental Distribution and Fate

Distribution

The distribution of isophorone in a "unit world" was calculated according to the Mackay fugacity model level I (V. 2.11) (UBA, 2002), based on the physico-chemical properties listed in section 1. The main target compartments were estimated to be water (87.6 %) and air (11.7 %), whereas soil and sediments (both 0.3 %) are expected to be of minor importance.

The distribution of isophorone between aqueous solutions and air can be calculated from water solubility and vapour pressure. Using a solubility of 14.5 g/l and a vapour pressure of 40 Pa (20°C), a Henry's law constant of 0.38 $Pa \cdot m^3/mol$ is obtained, indicating a slow volatilization from aqueous solution according to the criteria of Thomas (1990). For a model river (flow 1 m/sec) the volatilization half-life was calculated to 7.5 –11 days (WHO, 1995).

The distribution between the organic phase of soil or sediment solids and porewater can be calculated from the octanol/water partitioning coefficient. Using a log Kow of 1.67 and the equation log Koc = 0.52 log Kow + 1.02 (EC, 1996) a Koc value of 77 l/kg is calculated, indicating a low sorption potential to soil organic matter according to the criteria of Blume (1990).

Abiotic Degradation

In the atmosphere, isophorone is rapidly photodegraded by reaction with ozone and hydroxyl radicals. In a smog-chamber experiment, McQuaid et al. (2002) determined the reaction rate with OH-radicals to be 2.4×10^{-11} $cm^3 \times molecule^{-1} \times s^{-1}$. Based on a tropospheric OH-radical concentration of 500,000 molecules x cm^{-3} the corresponding half-life is calculated to 16 h. The reaction with tropospheric ozone is supposed to be of higher importance. A model calculation resulted in a reaction rate of 5×10^{-16} $cm^3 \times molecule^{-1} \times s^{-1}$. Based on an ozone concentration of 1×10^{12} $molecules \cdot cm^{-3}$, the reaction half-life is calculated to be 23 minutes.

Isophorone is not expected to undergo rapid direct photolytical degradation in the hydrosphere because of the lack of a strong chromophore. Furthermore, hydrolytic degradation is not to be expected.

Biodegradation

In a DOC-Die Away Test (similar to OECD 301 A) using isophorone as test substance 95 % degradation was determined after 28 days incubation (Huels AG, 1992). Activated sludge from a biological treatment plant receiving primarily municipal sewage was used as inoculum. The degradation curve reveals that about 80 % of the initial test substance concentration are degraded within 10 days after a lag phase of about of 8 days. Therefore, isophorone can be classified as readily biodegradable.

The inherent degradability of isophorone was studied by Huels AG (1996a). In a Zahn-Wellens Test similar to the OECD guideline 302 B, test substance degradation was 5 % after 3 days (DOC determination) and 89 % after 14 days.

In a Coupled Units Test according to OECD 303 A, a degradation rate of 69 ± 11 % was found (mean of 24 measurements). Isophorone was employed in a concentration of 10.4 mg DOC/l, the mean retention time was 3 hours (Huels AG, 1997).

In several further tests on ready biodegradability (e.g. MITI, 1992) only poor degradation rates were observed. In these tests, the initial isophorone concentration was 100 mg/l. Tests on toxicity of isophorone on microorganisms (cf. section 4.1.4) indicate that inhibiting effects were observed at this concentration range. Therefore, these tests are considered to be not valid and are, consequently, not used for the assessment.

The available experimental results from valid standard degradation tests reveal that isophorone is completely mineralizable. In the DOC-Die Away Test the pass level (> 70 % DOC removal within 10 days) was reached, therefore the substance can be classified as readily biodegradable.

Tests on biodegradation under anaerobic conditions are not available.

Bioaccumulation

The bioaccumulation of isophorone in fish (*Cyprinus carpio*) was determined in a test according to OECD guideline 305 C (MITI, 1992). BCFs in the range of 1.1 - 1.8 l/kg with 0.5 mg isophorone/l resp. <10 l/kg with 0.05 mg/l were reported, indicating no significant bioaccumulation potential. The variation of results may be partially explained by the variation of the lipid content in exposed fish (2 - 6 %).

Veith et al. (1980) determined the bioconcentration of ^{14}C-labelled isophorone in the fish *Lepomis macrochirus*. The organisms were exposed to a 92.4 µg/l solution of the tests compound. After a test period of 14 days, a BCF value of 7 l/kg was obtained. The determination of radioactivity includes possible metabolized and/or incorporated intermediates. After the fish were transferred into pollutant-free water, a depuration half-life of 1 day was determined.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Absorption

Isophorone is well absorbed via the oral and inhalative route (Dutertre-Catella, 1976). Already 10 minutes after oral administration of 1 g ^{14}C isophorone/kg to 2 rabbits, remarkable isophorone concentrations in the blood (0-102 µg/ml) were detected, which started to decrease from 75 - 141 µg/ml after 30 minutes and 88 - 94 µg/ml after 1 hour down to less than 0.5 µg/ml at 21 hours post treatment (Dutertre-Catella, 1976). Good dermal absorption can be concluded from the systemic effects in the acute toxicity studies (see chapter 3.2.3).

Distribution

In male and female rats and rabbits isophorone is rapidly distributed. One hour after a single oral administration (4 g/kg) of ^{14}C isophorone the highest concentrations were found in the stomach, pancreas, adrenals, spleen and liver of rats and rabbits. After inhalation (400 ppm) for 4 hours, the highest concentrations were obtained in the kidney, adrenals, liver, pancreas and brain of rats immediately after the termination of inhalation (Dutertre-Catella, 1976). 24 hours after the last oral administration of 500 mg radioactive labeled isophorone/kg for 8 consecutive days to mice and rats, the highest concentrations were found in kidney, liver, lung, spleen and adrenals (Thier, 1991). 48 hours after oral administration of 1 g/kg isophorone to male and female rats, only traces of isophorone could be determined in the stomach and no isophorone was measured in the other organs (Dutertre-Catella, 1976).

Metabolism

The metabolic pathway of isophorone is described in figure 1. After oral application of 1 g isophorone (3,5,5-trimethyl-2-cyclohexene-1-one) [1] /kg bw, one main metabolite in rats and rabbits is 5,5-dimethyl-1-cyclohexene-3-one-1-carboxylic acid [2]. This metabolite is formed by oxidation of the 3-methyl group of isophorone and then glucuronidated (Dutertre-Catella et al., 1978; Truhaut et al., 1970). Further metabolites may be formed through hydrogenation at the 1-one or/and 2-ene-position or after further oxidation processes. Dihydroisophorone (3,5,5-trimethyl-cyclohexanone) [3], isophorol (3,5,5-trimethyl-2-cyclohexen-1-ol) [4] and 3,5,5-trimethylcyclohexan-1-ol (cis- and trans-isomer) [5], 6-Oxoisophorone (3,5,5-trimethyl-2-cyclohexen-1,6-dione) [6], 4-Oxoisophorone (3,5,5-trimethyl-2-cyclohexen-1,4-dione) [7], 4-hydroxy-isophorone (4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one) [8] and 6-hydroxyisophorone (6-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one) [9] were identified via GC/Kovats indices and GC/MS (Dutertre-Catella et al., 1978; Thier, 1991).

Isophorol is eliminated as glucuronide (Dutertre-Catella et al., 1978; Truhaut et al., 1970). Dihydroisophorone [3] is mainly found in the urine of rats, while rabbits produce primarily isophorol [4] (Dutertre-Catella et al., 1978). The alcohols isophorol [4] and 3,5,5-trimethyl-cyclohexan-1-ol [5] could not be detected in the urine of male rats after repeated dose oral isophorone administration (Thier, 1991).

The following metabolic pathway scheme was proposed (Greim, 1995):

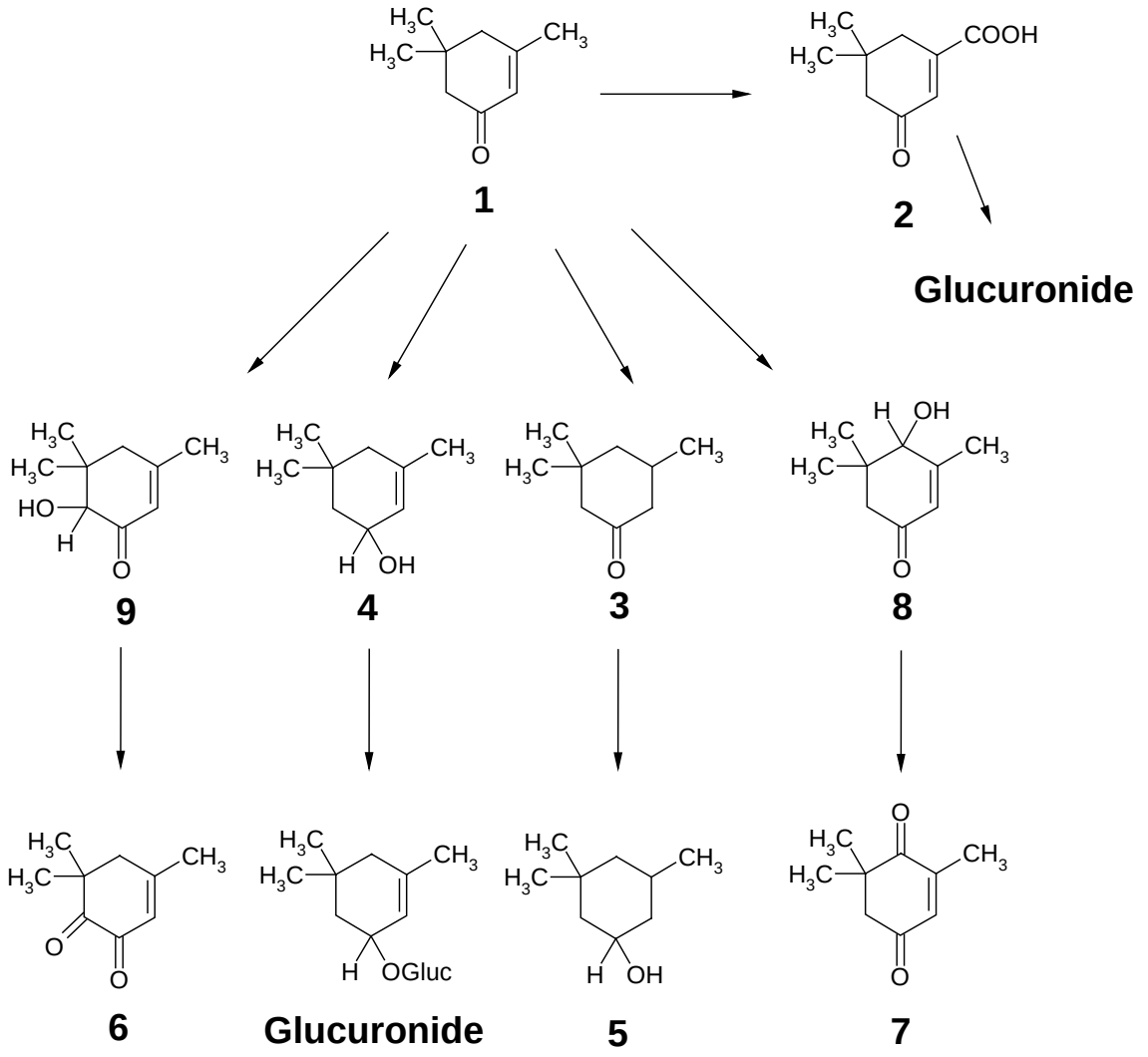


Figure 1

There are data to suggest that the glutathione system is also involved in the metabolism of isophorone. After i.p. administration of 500 mg isophorone/kg to sexually matured rats the glutathione depletion in liver, testes and epididymis was measured. In the liver 40 % reduction of the glutathione content was determined (maximum: 4 h after application), 82 % reduction was measured in testes (maximum: 4 h after application) and 72 % in the epididymis (maximum: 8 h after application) (Gandy et al., 1990).

Excretion

After administration of 400 ppm isophorone to rats (4 h), a part of the isophorone was expired unchanged (Dutertre-Catella, 1976). Also in the urine of orally treated rabbits and rats unreacted isophorone could be isolated (Dutertre-Catella et al., 1978; Truhaut et al., 1970; Thier, 1991). After single as well as repeated-dose oral administration of isophorone to rats 80 % of radioactivity was excreted within 96 h. 50 - 65 % were detected in the urine (Thier, 1991).

Conclusion

Conclusion: Upon oral and inhalative administration, isophorone is well absorbed and rapidly distributed through the body of rats and rabbits. While part of the absorbed isophorone is excreted unchanged via urine and exhaled air, metabolites are mainly excreted as glucuronides. The tendency of isophorone to bioaccumulate is very low, since within 24 hours after administration more than 93% of orally administered isophorone was excreted by rats.

3.1.2 Acute Toxicity

Oral

LD_{50} values of isophorone in rats were 1500 mg/kg bw (Günzel and Richter, 1968a), 2100 mg/kg bw (Dutertre-Catella, 1976) and 3450 mg/kg bw (Esso Research, 1964) The LD_{50} in mice is 2200 mg/kg bw (Dutertre-Catella, 1976). Clinical signs like general apathy, depression, weariness (leading to coma) ptosis, lacrimation and laboured respiration occurred at doses of ≥ 1450 mg/kg bw (Günzel and Richter, 1968a, Dutertre-Catella, 1976, Esso Research, 1965b). At doses of ≥ 5000 mg/kg bw congestion of lungs, kidneys and pancreas (Esso Research, 1964) and liver lesions (Dutertre-Catella, 1976) were found at necropsy.

Inhalation

In acute inhalation studies isophorone showed very low acute toxicity with LC_{50} values sometimes exceeding the maximum tested concentrations (> 3500 and = 7000 mg isophorone/m^3) in rats, mice and guinea pigs (Esso Research 1964, Esso Research, 1965b). Mortality was observed in another study with rats but not guinea pigs at concentrations ≥ 10,570 mg/m^3 (Smyth and Seaton, 1940). Clinical signs were nose and eye irritation, accelerated, laboured respiration, intestinal peristalsis and coma at dose ≥ 5000 mg/m^3 (Smyth and Seaton, 1940, Esso Research, 1965b) At necropsy of the high exposure concentrations, congestion of the lungs and occasionally observed liver and stomach congestion was found (Esso Research, 1965b; Smyth and Seaton, 1940).

Dermal

In rats, the LD_{50} after dermal application was 1700 mg/kg bw (Günzel and Richter, 1968b). For rabbits the LD_{50} values were 1200 mg/kg bw (Dutertre-Catella, 1976) and > 3160 mg/kg bw (Esso Research, 1964). Clinical signs were general apathy, later on occasionally coma, cachexia, tremor, lacrimation (Günzel and Richter, 1968b) and depression, accelerated/labored respiration, sprawling, prostration and narcosis at doses of 3160 mg/kg bw at least. At necropsy uniform thickening of the cutaneous stomach mucosa and pulmonary emphysema, edema or hyperemia was observed.

Conclusion

Conclusion: The acute toxicity in laboratory animals is low to moderate (oral LD_{50} ≥ 1500 mg/kg bw; dermal LD_{50} ≥ 1200 mg/kg bw; inhalative LC_{50} = 7000 mg/m^3).

3.1.3 Irritation

Skin Irritation

One guideline study was performed on rabbits with 0.5 mg (corresponds to 1500 mg isophorone/kg bw) and did not result in significant irritancy (Potokar et al., 1985). Only slight irritations were seen after application of 0.5 ml according to a modified Draize protocol (24 hrs exposure) (Dutertre-Catella, 1976). After application of 200 mg/kg isophorone (24 hrs exposure) only slight erythema

and desquamation during the first four days after 4 hours of exposure was observed (Esso Research, 1964). The findings were fully reversible.

Eye Irritation

Two Draize tests were performed on each 6 male and female rabbits. When applied undiluted to rabbits' eyes isophorone is moderately to severely irritating to the eyes (Esso Research, 1964; Truhaut et al., 1972). In one study marked irritation was observed which reversed within 14 days but was still present after 7 days (Esso Research, 1964), while in the other study, irritant scores of 14/80 (cornea), 0/10 (iris) and 6/20 (conjunctiva) were reported and had reversed within 7 days (Truhaut et al., 1972).

Conclusion: Isophorone is an eye irritant and a respiratory irritant but does not irritate the skin.

3.1.4 Experience with Human Exposure

Only few human data are available. The irritating potential of isophorone was determined in two investigations with 6 and 12 volunteers respectively. After exposure to 100 up to 230 mg/m^3 isophorone for a few minutes, throat irritation was reported at $\geq$ 199 mg/m^3 and eye and nasal irritation at concentration of $\geq$ 230 mg/m^3 (Esso Research, 1965a; Smyth and Seaton, 1940). In a further investigation 12 volunteers were exposed to isophorone vapors for 15 minutes. At 144 mg/m^3 chamber concentration eye, nose and throat irritation were reported (Silverman et al., 1946). At higher concentrations (1150 and 2300 mg/m^3) a few complaints of nausea, headache, dizziness, faintness, inebriation and a feeling of suffocation were reported (Smyth and Seaton, 1940).

The odor threshold of isophorone in air is 0.20 ppm (v/v) = 1.15 mg/m^3 (Amoore and Hautala, 1983). 100 % odor detection was found at 100 mg/m^3 (Esso Research, 1965a). 40 % of the exposed subjects objected isophorone odor at a concentration of 58 mg/m^3 (10 ppm), and this concentration was judged to be the highest tolerable air level for 8 hour exposure (Silverman et al., 1946). The current MAK value is 11 mg/m^3 (2 ppm) (Greim, 1995).

The 7-minute airborne exposure NOAEL and LOAEL for eye irritation in humans are 199 and 359 mg/m^3, respectively (Esso Research, 1965a).

Conclusion: The odor threshold of isophorone is about 1.15 mg/m^3, irritating effects in eyes, nose and throat are observed after 15 minutes exposure to concentrations above 144 mg/m^3. The substance is classified in Europe as an irritant to eyes and respiratory system.

3.1.5 Sensitisation

In a guinea pig maximization test (according to OECD Guideline 406, positive control not required by 1981 guideline version), sensitization was not observed in any of 20 animals (Huels AG, 1988a).

Conclusion: Isophorone is not sensitizing in animal studies.

3.1.6 Repeated Dose Toxicity

Inhalation

Results of studies with exposure by inhalation are given in Table 3.1. Studies are available for rats, mice, rabbits, and guinea pigs.

In rats exposed for 4 weeks to 208 mg/m^3 isophorone reduced body weights in males, changes in haematological parameters and reduced liver weights in males and females were found (Exxon, 1968).

In a study with 6 weeks duration, at doses ≥ 287 mg/m^3 congested kidneys, dilated Bowman's capsules and lung changes (irritation, congestion) were found in rats and in guinea pigs (Smyth et al., 1942). Further findings observed in this study were blood cell changes and albuminuria at doses ≥ 575 mg/m^3 and eye and nasal irritations at 2874 mg/m^3. Eye and nose irritations have also been observed in a more recent study in Wistar rats and New Zealand rabbits at 1436 mg/m^3 after 18 months exposure. In addition at this concentration slightly increased microvacuolization of the livers was observed (Dutertre-Catella, 1976).

No effects were found in histopathological examinations of the respiratory tract of Swiss mice after exposure to 164 and 513 mg/m^3 for up to 14 days (Zissu, 1995).

Table 3.1: Results After Inhalational Administration of Isophorone

Species **Sex** **No of animals/ group**	**Dose (mg/m^3)**	**Exposure**	**Examinations, deviations from standard protocols**	**Target Organs Effects**	**Reference**
Rat Charles River Caesarian 10 m + 10 f	0, 208 (analytical control)	6 h/d 5 d/w 28 d	Organ weights, histopathology: only lungs, liver, kidneys, adrenals, spleen. No urinalysis	M: body weight gain ↓, abs. + rel. liver weight↓ m + f: lymphocytes ↑, haemoglobin ↑, neutrophils ↓	Exxon, 1968
Rat Wistar 10 m	0, 144, 287, 575, 1150, 2874 (analytical control)	8 h/d 5 d/w 6 w	No organ weights. Histopathology: only liver, kidney, spleen, lung, adrenal, heart muscle; some animals in addition muscle, pancreas, testes, small intestine	≥ 287 mg/m^3: kidney (congestion, dilatation of Bowman's capsule; cloudy swelling of convoluted tubular epithelium) ≥ 575 mg/m^3: mortality ↑ body weight gain ↓; lungs (irritation, congestion, capillary leakage and desquamation of epithelium) Blood cell changes (not further specified) albumin in urine ↑≥ 2874 mg/m^3, chronic conjunctivitis and nasal irritiation, sometimes proceeding to bloody exsudate	Smyth et al., 1942
Rat Wistar 10 m, 10 f	0, 2873 (analytical control)	6h/d 5 d/w 4 m (f) 6 m (m)	No organ weights, no clinical chemistry, urinalysis haematology, histopathology: only performed on males (lungs, liver): limited information	Mortality: f: 1/10, m: 2/10 m + f: Eye and nose irritation	Dutertre-Catella, 1972
Rat Wistar 10 m + 10 f Rabbit New Zealand white 2 m + 2 f	0, 1436 (analytical control)	6 h/d 5 d/w 18 m	No organ weights, no clinical chemistry, urinalysis hematology, histopathology: limited information	m + f: slight conjunctivitis, slight irritation of nasal mucosa Microvacuolization of livers	Dutertre-Catella, 1972
Guinea pig 10 (m + f)	0, 144, 287, 575, 1150, 2874 (analytical control)	8 h/d 5 d/w 6 w	No organ weights. Histopathology: Liver, kidney, spleen, lung, adrenal, heart muscle; some animals also voluntary muscle, pancreas, testicle, small intestine	The same findings were recorded as in rats (but not quantified, see above), guinea pigs were less sensitive	Smyth et al., 1942

Table 3.1 (cont.): Results After Inhalational Administration of Isophorone

Species Sex No of animals / group	Dose (mg/m^3)	Exposure	Examinations, deviations from standard protocols	Target Organs Effects	Reference
Mouse Swiss 10 m	0, 164, 513 (analy-tical control)	6 h/d 4, 9, 14 d	Only investigations: histopathology of trachea, lungs, nasal airway	No findings	Zissu, 1995

Oral

Results of studies with oral application of isophorone are presented in Table 3.2.

Male and female Fischer rats were administered 0, 125, 250, 500, 1000 and 2000 mg isophorone/kg bw/day in a 16 day and 0, 62.5, 125, 250, 500 and 1000 mg/kg bw/day in a 13 week investigation, the former being a dose finding study for the latter.

In the dose finding study, one of five males and four of five females that received 2000 mg/kg bw/day isophorone died. Effects at 1000 mg/kg bw/day were reduced body weight gains in male (-13.9%) and female rats (-6.7%).

In the 13-week study, one of ten females of the tope dose group died. In the 1000 mg/kg bw/day group reduced body weight gain was only seen in male rats (-5.1%). The NOAEL considering effects observed in both studies is 500 mg isophorone/kg bw/day for male and female rats (NTP, 1986).

In a guideline-comparable study with male and female CFE rats dosed with 750, 1000, or 3000 ppm isophorone via diet – corresponding to 57.0, 102.5, 233.8 mg/kg bw/day for males and to 73.9, 163.8 and 311.8 mg/kg bw/day for females – for 13 weeks, the only observed effect was a reduced body weight gain (-12 to -13%) in male rats at 3000 ppm (during weeks 6,7,8,9,10 and 11). The NOAEL derived from this study is 102.5 mg/kg bw/day for male and 311.8 mg/kg bw/day for female rats (Rohm & Haas, 1972b).

After administration of isophorone up to 2000 mg/kg bw/day to male and female B6C3F1 mice for 16 days, the reported effect were mortality at 2000 mg/kg and reduced body weight gains in male (max. -7.8%) and female (max. -9.3%) mice at lower dosages. In the 13 week study with dosages up to 1000 mg/kg bw/day, 3 of 10 females that received the top dose died. Final mean body weights for animals of each sex were not dose related. The NOAEL considering both studies is therefore 125 mg isophorone/kg bw/day for female (derived from the 16-day study) and 500 mg isophorone/kg bw/day for male mice (derived from the 16-day study) (NTP, 1986).

In a further guideline comparable 90-day study (Rohm & Haas Co., 1972a), beagle dogs (4 animals/dose/sex) were given orally gelatine capsules containing doses of 35, 75, or 150 mg isophorone per kg bodyweight. As the only minor clinical signs, incidences of soft stool were noted in the two upper dose levels.

NOAEL: $\geq$ 150 mg/kg bw/day for male and female beagle dogs.

Table 3.2: Results After Oral Administration of Isophorone

Species **Sex** **No of animals / group**	**Doses (mg/kg bw/day)**	**Duration**	**NOAEL (mg/kg bw/day)**	**Protocol (deviations from guideline)**	**Target Organs/Effects**	**Reference**
Rat Fischer 344 5 m, 5 f	0, 125, 250, 500, 1000, 2000 (gavage)	16 d	500	No organ weights, no hematology, clinical chemistry, or urinalysis	m, f: 2000 mg/kg bw/day: mortality ↑ ≥ 1000 mg/kg bw: body weight gain ↓	NTP, 1986
Rat Fischer 344 10 m, 10 f	0, 62.5, 125, 250, 500, 1000 (gavage)	13 w	m: 500 f: 500	No organ weights, no hematology, clinical chemistry, or urinalysis	m: 1000 mg/kg bw/day: body weight gain ↓ f: 1000 mg/kg bw/day: mortality ↑	NTP, 1986
Rat CFE 20 m, 20 f	M: 0, 57.0, 102.5, 233.8 F: 0, 78.9, 163.8, 311.8 (diet)	13 w	m: 102.5 f: ≥ 311.8	Organ weight: heart, liver, kidney, adrenals, thyroid, brain, testes	m: 233.8 mg/kg bw/day: body weight gain ↓	Rohm & Haas, 1972b
Mouse B6C3F1 5 m, 5 f	0, 125, 250, 500, 1000, 2000 (gavage)	16 d	m: 500 f: 125	No organ weight, no hematology, clinical chemistry, or urinalysis	f: ≥ 250 mg/kg bw: body weight gain ↓ m: ≥ 1000 mg/kg bw/day: body weight gain ↓ m, f: 2000 mg/kg bw/day: mortality ↑	NTP, 1986
Mouse B6C3F1 10 m, 10 f	0, 62.5, 125, 250, 500, 1000 (gavage)	13 w	m: ≥ 1000 F: 500	No organ weights, no hematology, clinical chemistry, or urinalysis	f: 1000 mg/kg bw/day: mortality ↑	NTP, 1986
Dog Beagle 4 m, 4 f	0, 35, 75, 150 (gelatine capsules)	13 w	≥ 150	Organ weight: heart, liver, kidney, spleen, thyroid, brain, testes	No findings	Rohm & Haas, 1972a

Conclusion

In subchronic studies, oral administration of high doses of isophorone (NOAEL (male rat, 90 days) = 102.5 mg/kg bw/day, NOAEL (female rat, 13 weeks) 500 mg/kg bw/day; NOAEL (male mouse, 16 days) = 500 mg/kg bw/day, NOAEL (female mouse, 16 days) = 125 mg/kg bw/day; NOAEL (dog, 90 days) ≥ 150 mg/kg bw/day) caused no significant toxic effects (all NOAELs are based on slight (<14%) reductions in body weight gain). After inhalational administration nose and eye irritation and blood and liver changes were observed (NOAEL (rat, 28 days) < 208 mg/m^3).

3.1.7 Mutagenicity

In vitro Studies

Table 3.3: Results of Different Tests on *in vitro* Genotoxicity of Isophorone

Type of test	System/ Strain	Conc. tested	Result *		Cytotoxicity, Comment	Reference
Ames Tests	TA 98, 100, 1535, 1537	TA 100, 1535 : 0 - 10,000 µg/plate TA 98, 1537: 0 -3333 µg/plate	+/- S9: negative		- S9 : 1000 µg/plate + S9 : 10,000 µg/plate	NTP, 1986
	TA 1535, 1537, 1538	0, 1, 10, 100, 1000 µg/plate	+/- S9: negative		No data	Hossack et al., 1978a
	TA 98, 100, 1535, 1537, 1538	0, 10 -5000 µg/plate	+/- S9: negative		No data	Huels AG, 1988b
Mammalian tests						
Mouse lymphoma test	L5178Y	-S9: 119.6 - 1196 µg/ml +S9: 81.9 - 818.8 µg/ml	+/- S9: negative		-S9: 1656 and 2208 µg/ml +S9: 1104 and 1472 µg/ml	O'Donoghue et al., 1988
Mouse lymphoma test	L5178Y	50 - 1600 µg/ml	+ S9: n.d.	- S9: positive	1600 µg/ml	McGregor et al., 1988 NTP, 1986 Tennant et al., 1987
Mouse lymphoma test	L5178Y	No data (3 - 6 hr treatment)	+ S9: ambi-gous (positive in one lab)	- S9: negative	Tested up to 10 - 20% RS (relative survival)	Honma et al., 1999a
Mouse lymphoma test	L5178Y	0 - 1500 µg/ml (24 h treatment)	+ S9: n.d.	- S9: positive (1300 µg/ml)	1500 µg/ml	Honma et al., 1999b
Chromosomal aberration	CHO	-S9: 50 - 1600 µg/ml, +S9: 250 - 1500 µg/ml	+ S9: negative	- S9: negative	5000 µg/ml	Gulati et al., 1989 Tennant et al., 1987 NTP, 1986
Chromosomal aberration (modified procedure)	CHL	750 -1750 µg/ml	+S9: positive (1250 µg/ml)	-S9: positive (1750 µg/ml)	+S9: 1750 µg/ml -S9: no data	Matsuoka et al., 1996

Table 3.4: Results of Different Tests on *in vitro* Genotoxicity of Isophorone

Type of test	System/ Strain	Conc. tested	Result *		Cytotoxicity, Comment	Reference
Sister chromatid exchange	Chinese hamster ovary cells	5 - 1600 µg/ml (+/- MA)	+ S9: negative	- S9: positive (≥ 500 µg/ml)	≥ 500 µg/ml	Tennant et al., 1987, Gulati et al., 1989 NTP, 1986
Unscheduled DNA synthesis	Primary rat hepatocytes	0.005 – 0.4 µl/ml	Negative		0.4 µl/ml	O'Donoghue, 1988
Transformation assay	BALB/c-3T3 cells	46 - 738 mg/l	Positive		716 mg/l	Matthews et al., 1993

* Concentrations are given, at which a positive reaction was observed, in the case of no concentration indication, data were not available.

Several Ames tests with *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 with and without S9 were negative (Mortelmans et al., 1986; NTP, 1986; Hossack et al., 1978a; Huels AG, 1988b).

The mouse lymphoma tests were primarily negative. In the presence of metabolic activation only one positive result was obtained (Honma et al., 1999a). Without metabolic activation some studies gave negative results (O'Donoghue et al., 1988, Honma et al., 1999a), others positive results (McGregor et al., 1988). Positive results were found only at reduced RTG (relative total growth) values (McGregor et al., 1988). In one study, the incubation time was increased to 24 h to render the test system especially sensitive for the detection of clastogens and spindle poisons. In this study, isophorone was positive in one of two trials (Honma et al., 1999b).

In a cytogenetic assay with Chinese Hamster Ovary (CHO) cells, no significant increase in chromosomal aberrations was observed (Gulati et al., 1989; NTP, 1986). A further chromosomal aberration assay performed on Chinese hamster lung (CHL) cells was positive with isophorone with metabolic activation only after a modified treatment of the cells (cells are treated for 6 h and then cultured in fresh medium for another 18 h (Matsuoka et al., 1996). Increased chromosome aberrations without metabolic activation were only observed at cytotoxic concentrations (Matsuoka et al., 1996).

Gulati et al. (1989) reported a significant increase in SCE frequency at concentrations of 500 - 1000 mg/l induced by isophorone only in the absence of S9 mix (no increase in the presence of Aroclor 1254-induced rat liver S9 mix). As these high isophorone concentrations were cytostatic, increased SCE frequencies could only be detected after delayed harvest.

Isophorone was tested for the induction of unscheduled DNA synthesis (UDS) in rat primary hepatocytes. Concentrations ranged from 0.005 - 0.4 µl/ml, the highest concentration being toxic. No increase in the mean nuclear grain count (as compared to controls) or in the incidence of cells undergoing repair was detected at any dose level (O'Donoghue et al., 1988; Microbiological Associates, 1984b).

In vivo Studies

In a mouse micronucleus assay, 496.8 mg/kg (= LD_{20} = MTD) isophorone was administered i.p. to 5 male and 5 female CD-1 mice per group. Sampling time was 12, 24, 48 hrs post dosing. Significant increases in the number of micronucleated polychromatic erythrocytes (PCE) were not observed (O'Donoghue et al., 1988; Microbiological Associates, 1984a). Likewise, in a study with CFLP mice (5 animals/dose/sex; gavage doses of 450, 900, 1800 mg/kg given in 2 equal parts [i.e.

half of total dose] separated by an interval of 24 hrs) a negative result was obtained 6 hours after the last dosage of isophorone (Hossack et al., 1978b).

In a DNA binding study, male and female F344 rats and B6C3F1 mice (25 animals/group) were administered 500 mg/kg [1,3,5-^{14}C]-isophorone. Livers and kidneys (target organs in the NTP carcinogenicity study) were removed after 24 hrs. There was no binding of isophorone or its metabolites to DNA of these organs (Thier et al.,1990).

In a Sex Linked Recessive Lethal (SLRL) test with *Drosophila melanogaster*, fruit flies were fed a diet containing 2000 mg/kg isophorone. After 72 hrs of exposure, surviving males were mated. As feeding exposure was found to be non-mutagenic, 2 - 3 day old males were then injected with a 0.7 % NaCl solution containing 12,500 mg/l isophorone. 24 hrs post injection, males were mated. Again there was no indication of a mutagenic effect (Foureman et al., 1994).

Conclusion

Conclusion: Although in one standard mouse lymphoma assay a positive result was observed, the majority of in vitro genotoxicity studies revealed clearly negative results. Together with the negative in vivo results and the negative DNA binding assay, the overall conclusion is that isophorone is not mutagenic.

3.1.8 Carcinogenicity

In an NTP 2-year study (NTP, 1986) Fischer-344 rats (50 animals/dose/sex) were exposed by gavage to daily doses (5 days/week) of 0, 250, or 500 mg/kg isophorone in corn oil. The overall survival rate was low (males 33/50, 33/50, 14/50; females 30/50, 23/50, 20/50). Increased mortality was observed after week 98 in males in the 500 mg/kg bw dose group. Compound related clinical signs were not observed. Throughout the study, the mean body weights of high dose males averaged 5 % lower than vehicle controls. During the second year, the mean body weights of high dose females averaged 8 % lower than vehicle controls.

Dosed males showed proliferative lesions of the kidney (tubular cell hyperplasia: 0/50, 1/50, 4/50; tubular cell adenoma: 0/50, 0/50, 2/50; tubular cell adenocarcinoma: 0/50, 3/50, 1/50; epithelial hyperplasia of the renal pelvis: 0/50, 5/50, 5/50) and an increased mineralization of the medullary collecting ducts (1/50, 31/50, 20/50). Low dose males revealed more severe nephropathy than is commonly observed in aging F344 rats. Female rats only showed a statistically significant increase in nephropathy (21/50, 39/50, 32/50) with no further findings in the kidneys.

Carcinomas of the preputial gland were increased in high dose males only (0/50, 0/50, 5/50). The preputium is only investigated histopathologically when gross lesions are found, therefore neither true tumor incidences from this study nor from historical controls are available. As also two clitoral gland tumors were found in low dose females, a treatment related effect cannot be discounted.

Investigations on α2u-globulin induced nephropathy:

Various chemicals are known to induce nephropathy in male rats only. Only 60 % of α2u-globulin is reabsorbed in the kidney of male rats. 40 % of this low molecular weight protein remains in the filtrate and is excreted to the urine. The amount of α2u-globulin in female rats is 120 times lower and the protein is nearly absent in mice and in men. Chemicals that induce α2u-globulin nephropathy bind to the protein in the liver of the animals; these conjugates are difficult to hydrolyse and induce the formation of hyaline droplets which accumulate in the tubules (Charbonneau and Swenberg, 1988; Swenberg et al., 1989).

Isophorone as well as the proposed metabolites isophorol and dihydroisophorone form protein complexes with α2u-globulin in vivo, (SDS-page and immunoblotting after 14 d isophorone administration; GC/MS determination of isolated protein complexes) (Saito et al., 1992; Strasser et al., 1988). The degradation of α2u-globulin via lysosomal proteinases was decreased by 33 % (Lehman-McKeeman et al., 1990). In a further experiment with male NCI-Black-Reiter rats (strain is unable to synthesize the hepatic form of the low molecular weight protein α2u -globulin) isophorone administration, which clearly induced kidney lesions in male F344 rats, failed to induce nephropathy, α2u-globulin or the formation of hyaline droplets (Dietrich and Swenberg, 1991). It is thus concluded that the observed nephropathy in male rats is caused via the α2u-globulin mechanism and therefore irrelevant to other species.

In a parallel NTP 2-year gavage study with B6C3F1 mice (50 animals/dose/sex) animals were dosed daily (5 days/week) with 0, 250, or 500 mg/kg isophorone in corn oil. The survival of male mice was low (13/50, 13/50, 18/50) in contrast to female mice, where there was a trend towards increased survival of dosed animals relative to the controls (24/50, 33/50, 34/50). In high dose males, there was an increased incidence of hepatocellular adenomas and carcinomas (18/48, 18/50, 29/50). Increased incidences of coagulative liver necrosis (3/48, 10/50, 11/50) and hepato-cytomegaly (23/48, 39/50, 37/50) were found in low and high dose males.

Mesenchymal tumors of the integumentary system (6/48, 8/50, 14/50) were increased in high dose males. An increased incidence of lymphomas or leukemias was noted in low dose males only (8/48, 18/50, 5/50). These results were interpreted as chemically related marginal increases in number of neoplasms (equivocal evidence).

Compound related non-neoplastic or neoplastic lesions associated with isophorone exposure were not seen in female mice.

Conclusion

There was some evidence of carcinogenicity of isophorone in male rats (kidney tumors, preputial gland carcinomas). The kidney tumors can be attributed to an α2u-globulin associated mechanism. The observed nephropathy in male rats is therefore irrelevant to other species. As the preputium is only investigated histopathologically when gross lesions are found, neither true tumor incidences from this study nor from historical controls are available. Therefore, the higher incidence of preputial gland tumors in high dose male rats cannot be put into perspective. There was equivocal evidence of carcinogenicity for male mice (liver tumors, mesenchymal tumors of the integumentary system). There was no evidence of carcinogenicity of isophorone in female rats and mice.

3.1.9 Toxicity to Reproduction

In a limited one generation study, 10 male and 10 female Wistar rats were exposed to 2872 mg/m^3 (500 ppm) isophorone in air (Dutertre-Catella, 1976). After three months of exposure, 5 exposed males each were mated with 5 control and 5 exposed females, 5 control males each were mated with 5 control and 5 exposed females. Exposure of females continued throughout gestation and they were allowed to deliver. Treatment with isophorone did not influence pregnancy rates and litter sizes nor were there any abnormalities observed in the pups.

The histological examinations of the reproductive organs of male and female mice and rats (mammary gland, seminal vesicle, prostate/testis or ovary/uterus) treated orally with up to 1000 mg isophorone/kg bw for 13 weeks did not reveal any adverse effects after macroscopic and microscopic examination (NTP,1986). In a 90 days study with male and female beagle dogs (4 animals/dose/sex were administered up to 150 mg isophorone per kg bw per day) no changes were

reported either after histopathological examination of testes, prostate, seminal vesicles and ovary, uterus, mammary gland respectively (Rohm & Haas Co., 1972a).

Conclusion

There is no evidence indicating that isophorone interferes adversely with the reproduction. No changes were observed in pregnancy rates, litter sizes, pups abnormalities or in histopathological examinations of the reproduction organs after long-term studies.

3.1.10 Developmental Toxicity

Pregnant Fischer 344 rats and CD-1 mice were exposed on days 6 through 15 of gestation to isophorone concentrations of 144, 289, or 664 mg/m³ (22 animals per dose level). There was a significant reduction in food consumption in rats of the highest dose group. Body weight was reduced in rats (gestation day 12: -6.1%; gestation day 15: -6.8%) and mice (gestation day 18, corrected for uterine weight: -5.6%) of the highest dose group. In rats, a dose related increase in alopecia was observed, as well as a discoloration of the cervical and anogenital region. In mice, this effect was observed only in one animal of the high dose group. Adverse effects on the fetuses were not observed (Exxon,1984).

Conclusion

In inhalation teratogenicity studies with rats and mice, the NOAELs for maternal toxicity were 289 mg/m³ (based on <7% reductions in body weight gains). Isophorone was neither embryotoxic nor teratogenic up to the highest test concentration of 664 mg/m³ isophorone.

3.2 Initial Assessment for Human Health

Upon oral and inhalative administration, isophorone is well absorbed and rapidly distributed through the body of rats and rabbits. While part of the absorbed isophorone is excreted unchanged via urine and exhaled air, metabolites are mainly excreted as glucuronides. The tendency of isophorone to bioaccumulate is very low, since within 24 hours after administration more than 93% of orally administred isophorone was excreted by rats.

The acute toxicity in laboratory animals is low to moderate (oral $LD_{50} \geq 1500$ mg/kg bw; dermal $LD_{50} \geq 1200$ mg/kg bw; inhalative LC_{50} = 7000 mg/m^3). Isophorone is an eye irritant and a respiratory irritant but does not irritate the skin. It is not sensitizing in animal studies.

In subchronic studies, oral administration of high doses of isophorone (NOAEL (male rat, 90 days) = 102.5 mg/kg bw/day, NOAEL (female rat, 13 weeks) 500 mg/kg bw/day; NOAEL (male mouse, 16 days) = 500 mg/kg bw/day, NOAEL (female mouse, 16 days) = 125 mg/kg bw/day; NOAEL (dog, 90 days) ≥ 150 mg/kg bw/day) caused no significant toxic effects (all NOAELs are based on slight (<14%) reductions in body weight gain). After inhalational administration nose and eye irritation and blood and liver changes were observed (NOAEL (rat, 28 days) < 208 mg/m^3).

Although in one standard mouse lymphoma assay a positive result was observed, the majority of in vitro genotoxicity studies revealed clearly negative results. Together with the negative in vivo results and the negative DNA binding assay, the overall conclusion is that isophorone is not mutagenic.

There was some evidence of carcinogenicity of isophorone in male rats (kidney tumors, preputial gland carcinomas). The kidney tumors can be attributed to an α2u-globulin associated mechanism. The observed nephropathy in male rats is therefore irrelevant to other species. As the preputium is only investigated histopathologically when gross lesions are found, neither true tumor incidences

from this study nor from historical controls are available. Therefore, the higher incidence of preputial gland tumors in high dose male rats cannot be put into perspective. There was equivocal evidence of carcinogenicity for male mice (liver tumors, mesenchymal tumors of the integumentary system). There was no evidence of carcinogenicity of isophorone in female rats and mice.

There is no evidence indicating that isophorone interferes adversely with the reproduction. No changes were observed in pregnancy rates, litter sizes, pups abnormalities or in histopathological examinations of the reproduction organs after long-term studies. In inhalation teratogenicity studies with rats and mice, the NOAELs for maternal toxicity were 289 mg/m³ (based on <7% reductions in body weight gains). Isophorone was neither embryotoxic nor teratogenic up to the highest test concentration of 664 mg/m³ isophorone.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

4.1.1 Fish

Acute Toxicity Test Results

Valid tests on acute toxicity to fish are available for 3 freshwater and 1 marine species. An overview is presented in table 4.1.

A flow-through test on the acute toxicity of isophorone to *Pimephales promelas* was conducted by Geiger et al. (1990). The fish were exposed in Lake Superior water to 5 test substance concentrations. Analytical measurements revealed that the isophorone concentrations were stable during the test period. Based on measured concentrations a 96h-EC50 of 217 mg/l was obtained. The affected fish lost schooling behavior, were hypoactive and under reactive to external stimuli and were darkly coloured. They had also spinal column deformities and lost equilibrium prior to death.

Similar results were obtained by Cairns and Nebeker (1982) in a comparable test with the same species. Depending on the fish age, 96h-EC50 values of 255 mg/l (6 - 8 weeks old) and 145 mg/l (3 weeks old) were obtained, indicating an increased sensitivity of earlier life stages.

The most sensitive fish in acute toxicity tests was the marine species *Cyprinodon variegatus*. A flow-through test in natural seawater resulted in a 96h-EC50 of 140 mg/l (Ward et al., 1981).

Table 4.1: Toxicity of Isophorone to Fish in Short-term Tests

Species	Test type	Exposure period	Effects [mg/l]	Remarks	Reference
Pimephales promelas (freshwater)	flow through	96 h	LC50 = 228 mg/l (e) EC50 = 217 mg/l (e)	fish 4 - 10 weeks old	Geiger et al. (1990)
	flow through	96 h	LC50 = 145 mg/l (n) LC50 = 255 mg/l (n)	fish 3 weeks old fish 6 - 8 weeks old	Cairns and Nebeker (1982)
Cyprinodon variegatus (marine)	flow through	96 h	LC50 = 140 mg/l (e)		Ward et al. (1981)
	static	96 h	LC50 = 170 - 300 mg/l (n)		Heitmuller et al. (1981)
Oryzias latipes (freshwater)	static or semi-static	48 h	LC50 = 340 mg/l (n)		MITI (1992)
Leuciscus idus melanotus (freshwater)	static	48 h	LC50 = 209 mg/l (n)		Huels AG (1996b)

(n): nominal concentration (e): effective concentration

Table 4.2: Toxicity of Isophorone to Fish in Long-term Tests

Species	Test type	Exposure period	Effects [mg/l]	Endpoint	Reference
Pimephales promelas (freshwater)	flow through; early life stage	35 d	NOEC = 11 mg/l (e)	weight	Cairns and Nebeker (1982)
	flow through; early life stage	32 d	NOEC = 4.2 / 15.6 mg/l (2 determinations; e)	weight	Lemke et al. (1983)
Cyprinodon variegatus (marine)	flow through; early life stage	28 d	NOEC = 80 mg/l (e)	length	Ward et al. (1981)

(e): effective concentration

Chronic Toxicity Test Results

The long-term toxicity of isophorone to fish was examined in several early life stage tests (cf. table 4.2).

A flow-through test using *Pimephales promelas* embryos (maximum 48 hours old) was conducted by Cairns and Nebeker (1982). The fish were exposed over a period of 35 days to 5 isophorone concentrations in well water and one control. The test indicated that survival was affected by 112 mg/l but not by 56 mg/l and lower. Observing the fork length, a NOEC of 19 mg/l and a LOEC of 30 mg/l were obtained, while the fish weight was affected by 19 mg/l and higher but not by 11 mg/l. Based on fish weight as the most sensitive endpoint the NOEC is determined to 11 mg/l.

Similar results were obtained in 2 flow-through tests using the same species (Lemke et al., 1983). After 32 days of exposure, NOECs for the endpoint fish weight of 4.2 (2^{nd} test) and 15.6 mg/l (1^{st} test) were obtained. The authors explained the deviation of the results with differences in the feeding regime. At the weekends the larvae were fed 2 times a day, during the weeks 3 times a day. As in the 1^{st} test the embryos hatched at weekend and were therefore fed only twice a day during the first two days growth in the control was lower than in the second test where the embryos hatched in the mid of the week and were therefore fed 3 times a day during the fist days. However, the difference in the results of the two tests is below a factor of 4 and within the normal uncertainty range of laboratory ecotoxicity tests.

An early life stage test using eggs from the salt water fish *Cyprinodon variegatus* performed in a flow-through system resulted in a 28d-NOEC (based on fish length) of 80 mg/l. Based on hatching success a NOEC of 156 mg/l was obtained (Ward et al., 1981).

4.1.2 Invertebrates

The acute toxicity of isophorone to *Daphnia magna* was determined in a static test conducted according to a U.S. EPA guideline. After 24 h of exposure, the LC50 was calculated to 430 mg/l, while after a period of 48 h a LC50 of 120 mg/l and a NOEC of 15 mg/l were obtained (LeBlanc, 1980).

In a comparable test following a guideline similar to OECD 202, 4 replicate samples with each 5 individuals of maximum 24 h old daphnids were exposed to 9 test substance concentrations and one control. Analytical control was not performed. Based on immobilization as the endpoint, the nominal EC0 was reported to be 90 mg/l and the EC50 to 254 mg/l (Huels AG, 1996c).

Table 4.3: Tests on Acute Toxicity of Isophorone to Invertebrates

Species	Test type	Exposure period	Effects [mg/l]	Reference
Daphnia magna *(freshwater)*	static	24 h	EC0 = 90 mg/l (n) EC50 = 254 mg/l (n)	Huels AG (1996c)
	static	24 h 48 h	LC50 = 430 mg/l (n) LC50 = 120 mg/l (n) NOEC = 15 mg/l (n)	LeBlanc (1980)
Mysidopsis bahia (marine)	static	96 h	LC50 = 12.9 mg/l (n)	WHO (1995)
Artemia salina (marine)	static	24 h	EC50 = 430 mg/l (n)	Price et al. (1974)

Effects: immobilization (n): nominal concentration

A higher toxicity is reported for the marine invertebrate *Mysidopsis bahia*, the 96h-LC50 being 12.9 mg/l. The test was conducted during a U.S. EPA project, for which no final report was written. The test results are cited in later peer-reviewed substance dossiers (e.g. U.S. EPA, 1980; WHO, 1995). No test protocol is available, thus this test can not be validated.

Although the available tests for invertebrates have been performed in open static systems without analytical monitoring of the test substance concentration, they are regarded as sufficient for the hazard assessment, as isophorone is only low to moderately volatile. For fish both static tests without analytical monitoring and flow-through tests with analytical monitoring are available. The results from these tests differ not by orders of magnitude, thus supporting the adequacy of the available data for invertebrates.

4.1.3 Aquatic Plants

The growth inhibition of isphorone on the freshwater alga *Scenedesmus subspicatus* was tested by Huels AG (1996d) according to a test procedure similar to OECD- Guideline 201. The algae were exposed to 5 concentrations between 125 and 1000 mg/l and one control. Based on nominal concentrations the 72h-EC10 value (based on biomass development) was determined to 64 mg/l and the EC50 to 475 mg/l.

Table 4.4: Toxicity of Isophorone to Aquatic Plants

Species	Exposure period	Effects [mg/l]	Endpoint	Reference
Scenedesmus subspicatus (freshwater)	72 h	EC10 = 64 mg/l (n) EC50 = 475 mg/l (n)	Biomass	Huels AG (1996d)
Champia parvula (marine)	14 d	NOEC = 29.9 mg/l (n)	growth	Thursby et al. (1985)

(n): nominal concentration

Thursby et al. (1985) tested the effects of isophorone on the marine red alga *Champia parvula*. Various biological endpoints, namely vegetative growth, formation of tetrasporangia (asexual reproduction) and production of cystocarps (sexual reproduction) were observed. After 14 d of exposure, the most sensitive endpoints were found to be growth of tetrasporophytes and number of tetrasporangia exhibiting a LOEC of 49.8 mg/l. The next lowest test concentration was 60 % of this value, thus the NOEC is calculated to be 29.9 mg/l.

Although the available tests for algae have been performed in open static systems without analytical monitoring of the test substance concentration, they are regarded as sufficient for the hazard assessment, as isophorone is only low to moderately volatile. For fish both static tests without analytical monitoring and flow-through tests with analytical monitoring are available. The results from these tests differ not by orders of magnitude, thus supporting the adequacy of the available data for algae.

Determination of PNECaqua

Results from long-term tests are available for fish and algae. For the most sensitive species, *Pimephales promelas*, NOECs from three early life stage tests are available that are in the same order of magnitude. Therefore, the geometric mean of these 3 NOECs is calculated resulting in a value of 8.9 mg/l. This mean NOEC is used as basic value for the derivation of the PNECaqua. According to the TGD an assessment factor of 50 is applied as long-term tests are available for two

trophic levels. With this, the Predicted No Effect Concentration (PNEC) for the aquatic compartment is calculated to 0.178 mg/l.

However, results from tests on acute toxicity to invertebrates indicate that species from this trophic level may be more sensitive to isophorone than fish or algae. For the marine invertebrate *Mysidopsis bahia*, a 96h-LC50 of 12.9 mg/l was found in the literature. Applying an assessment factor of 1000 (EC, 1996), a PNEC of 0.013 mg/l would be obtained. However, as no test protocol is available on this study, the test could not be validated and the result is not used for the hazard assessment.

Toxicity to Microorganisms

In a respiration inhibition test using activated sludge according to OECD 209 with isophorone as test substance, a 3 h-EC_{50} of 100 mg/l was obtained. An EC10 value was not reported (Yoshioka et al., 1986). Further tests conducted with *Pseudomonas putida* (Huels AG, 1996e) and the protozoa *Tetrahymena pyriformis* (Yoshioka et al., 1986) resulted in higher effect values (cf. table 4.5).

Table 4.5: Toxicity of Isophorone to Microorganisms

Species	Exposure period	Effects [mg/l]	Endpoint	Reference
Activated sludge, domestic (OECD 209)	3 h	EC50 = 100 mg/l	respiration	Yoshioka et al. (1986)
Pseudomonas putida	18 h	EC10 = 340 - 530 mg/l (2 tests)	growth	Huels AG (1996e)
Tetrahymena pyriformis	24 h	EC50 = 420 mg/l	growth	Yoshioka et al. (1985)

all effect values are based on nominal test substance concentrations

Applying an assessment factor of 100 to the result of the respiration test, the PNEC for microorganisms in biological treatment plants is determined to be 1.0 mg/l.

4.2 Terrestrial Effects

Krenek and King (1987) tested the phytotoxicity of isophorone as a solvent for pesticide formulations. In a greenhouse, four crops (cotton, soybean, corn, and wheat) where sprayed “over the top” with undiluted isophorone at a dose of 3.27 ml/m^2. The most sensitive species was found to be soybean: 4 hours after application 50 % of the leaves were damaged. After 56 h, all plants showed evidence of renewed growth and recovery from any phytotoxic effects. None of the plants died or failed to resume what appeared to be normal growth.

No studies could be identified where isophorone was applied directly to soil. Therefore a PNEC for terrestrial organisms cannot be determined.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

According to a Mackay Level I model calculation the main target compartments for isophorone will be the hydrosphere (87.6 %) and atmosphere (11.7 %). The calculated Henrys’ law constant

indicates evaporation from surface waters within 7.5 to 11 days. With a calculated Koc of 77 l/kg the sorption potential to soil or sediments is expected to be low.

In the atmosphere, isophorone is rapidly removed by reaction with ozone with an estimated half-life of 23 minutes. The calculated half-life for photodegradation by reaction with OH radicals is 16 h.

Isophorone can be considered to be readily biodegradable. In surface waters, the main removal mechanisms are expected to be biodegradation and volatilization. Photolytical degradation in surface waters is expected to be of minor importance. Furthermore, hydrolytic degradation is not to be expected. Experimentally determined BCF values below 10 l/kg indicate a low bioaccumulation potential.

The lowest valid acute test results of aquatic testing determined for fish, daphnia, algae and bacteria were as following:

Cyprinodon variegatus: 96h-LC50 = 140 mg/l

Daphnia magna: 48h-EC50 = 120 mg/l

Scenedesmus subspicatus: 72h-EbC50 = 475 mg/l; 72h-EbC10 = 64 mg/l

Activated sludge: 3h-EC50 = 100 mg/l

In 3 fish early-life-stage tests with *Pimephales promelas* NOEC-values of 4.2 mg/l (32 d), 15.6 mg/l (32 d) and 11 mg/l (35 d) were obtained for the endpoint growth (measured as weight). The geometric mean of these 3 NOEC is 8.9 mg/l. Based on this value, a PNEC of 0.178 mg/l is calculated using an assessment factor of 50.

Based on a test to respiration inhibition, the PNECmicroorg. was determined to be 1.0 mg/l.

For the terrestrial compartment, a PNEC could not be calculated. Isophorone spiked into soils is expected to be rapidly removed by biodegradation, thus the test substance concentration will be unstable during the test period.

5 RECOMMENDATIONS

The substance is currently of low priority for further work based on its low hazard potential. The substance is an eye irritant. Although this does not warrant further work, this property should nevertheless be noted by chemical safety professionals and users.

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I U C L I D

Data Set

Existing Chemical	:	ID: 78-59-1
CAS No.	:	78-59-1
EINECS Name	:	3,5,5-trimethylcyclohex-2-enone
EC No.	:	201-126-0
TSCA Name	:	2-Cyclohexen-1-one, 3,5,5-trimethyl-
Molecular Formula	:	C9H14O

Producer related part

Company	:	Degussa AG
Creation date	:	20.12.2002

Substance related part

Company	:	Degussa AG
Creation date	:	20.12.2002
Status	:	
Memo	:	Submission to OECD (ICCA)
Printing date	:	11.09.2003
Revision date	:	31.05.2003
Date of last update	:	21.07.2003
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	cooperating company
Name	:	Degussa AG
Contact person	:	Dr. Michael Weiss, Marl
Date	:	01.03.2001
Street	:	Bennigsenplatz 1
Town	:	40474 Duesseldorf
Country	:	Germany
Phone	:	+49 2365 49-4607
Telefax	:	+49 2365 49-7275
Telex	:	
Cedex	:	
Email	:	michael.weiss@degussa.com
Homepage	:	www.degussa.com
Remark	:	Contact point for any correspondence relating to the submission of this data set: Degussa AG CF-CO-PM-Environment, Health & Safety Dr. Michael Weiss Bau 1137, PB 16 D-45764 Marl Reporting History

Year	Activity	Company
1994	Reporting	Huels AG
1997	Update	Huels AG
1998	None	Creanova Spezialchemie GmbH
2000	Update	Degussa-Huels AG
2002	Update extra	Degussa AG
2003	Update	Degussa AG

Type	:	cooperating company
Name	:	BP Chemicals Ltd.
Contact person	:	D.A. King, Hull
Date	:	07.03.1994
Street	:	Buckingham Palace Road 76
Town	:	SW1 WOSU London
Country	:	United Kingdom
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

Type	:	cooperating company
Name	:	Elf Atochem S.A.
Contact person	:	J. Grevin / J. Bakes
Date	:	01.04.1994
Street	:	4, Cours Michelet
Town	:	F-92091 Paris La Defense 10
Country	:	France
Phone	:	+33 4900 7121

Telefax	:	+33 4900 7214
Telex	:	
Cedex	:	
Email	:	jacqueline.bakes@atofina.com
Homepage	:	

Type	:	cooperating company
Name	:	The Dow Chemical Company
Contact person	:	Timothy Cawley
Date	:	10.04.2002
Street	:	39 Old Ridgebury Rd
Town	:	06817-0001 Danbury, CT
Country	:	United States
Phone	:	+1-203-794-5003
Telefax	:	+1-203-794-3133
Telex	:	
Cedex	:	
Email	:	cawleytj@dow.com
Homepage	:	

(130)

Type	:	cooperating company
Name	:	Daicel Chemical Industries, Ltd.
Contact person	:	Dr. Tsuneo Baba
Date	:	14.12.2000
Street	:	
Town	:	671-1283 Aboshi, Himeji
Country	:	Japan
Phone	:	+81-792-74-4096
Telefax	:	+81-792-74-5831
Telex	:	
Cedex	:	
Email	:	ts_baba@daicel.co.jp
Homepage	:	

(20)

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

Type	:	manufacturer
Name of plant	:	Degussa AG
Street	:	Herzogstrasse 28
Town	:	44651 Herne
Country	:	Germany
Phone	:	+49 2325/68-3541
Telefax	:	+49 2325/68-3555
Telex	:	
Cedex	:	
Email	:	
Homepage	:	www.degussa.com

Type	:	manufacturer
Name of plant	:	The Dow Chemical Company
Street	:	Route 25
Town	:	25112 Institute, WV
Country	:	United States

Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

(130)

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance
Substance type : organic
Physical status : liquid
Purity : ca. 97.5 % w/w
Colour :
Odour :

Remark : Company (site): Dow Chemical Company, Institute, WV (USA)

(130)

Purity type : typical for marketed substance
Substance type : organic
Physical status : liquid
Purity : ca. 99.4 % w/w
Colour :
Odour :

Remark : Company (site): Degussa AG, Herne (Germany)
The value of the purity includes the beta isomer.

(24)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Isophorone

Alpha-Isophorone

Isoacetophorone

3,5,5-Trimethyl-2-cyclohexene-1-one

3,5,5-Trimethyl-2-cyclohexen-1-one

3,5,5-Trimethyl-2-cyclohexenone

1,1,3-Trimethyl-3-cyclohexene-5-one

Isooctaphenone

(15)

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No : 471-01-2
EC-No : 207-434-1
EINECS-Name : 3,5,5-trimethylcyclohex-3-en-1-one
Molecular formula :
Value : ca. 1.5 % w/w

Purity : typical for marketed substance
CAS-No : 87-99-0
EC-No : 201-788-0
EINECS-Name : xylitol
Molecular formula :
Value : ca. .15 % w/w

Purity : typical for marketed substance
CAS-No : 504-20-1
EC-No : 207-986-3
EINECS-Name : 2,6-dimethylhepta-2,5-dien-4-one
Molecular formula :
Value : ca. .12 % w/w

Purity : typical for marketed substance
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : water
Molecular formula :
Value : <= .1 % w/w

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name : trimethylcyclohexanone
Molecular formula :
Value : <= .06 % w/w

Purity	:	typical for marketed substance
CAS-No	:	14376-79-5
EC-No	:	238-350-3
EINECS-Name	:	3,3,5,5-tetramethylcyclohexanone
Molecular formula	:	
Value	:	ca. .05 % w/w

Purity	:	typical for marketed substance
CAS-No	:	
EC-No	:	
EINECS-Name	:	2,3,5,5-tetramethylcyclohexanone
Molecular formula	:	
Value	:	ca. .01 % w/w

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity	:	ca. 100000 - tonnes produced in
Remark	:	Estimated worldwide production

1.6.1 LABELLING

Labelling	:	as in Directive 67/548/EEC
Specific limits	:	yes
Symbols	:	Xi, , ,
Nota	:	, ,
R-Phrases	:	(21/22) Harmful in contact with skin and if swallowed (36/37) Irritating to eyes and respiratory system (40) Possible risks of irreversible effects
S-Phrases	:	(2) Keep out of reach of children (13) Keep away from food, drink and animal feeding stuffs (23) Do not breathe ... (36/37/39) Wear suitable protective clothing, gloves and eye/face protection (46) If swallowed, seek medical advice immediately and show this container or label
Remark	:	C >= 25 %: Xn, R21/22-36/37-40 25 % > C >= 10 %: Xn; R36/3 Index No. 606-012-00-8

1.6.2 CLASSIFICATION

Classified	:	as in Directive 67/548/EEC
Class of danger	:	carcinogenic, category 3
R-Phrases	:	(40) Possible risks of irreversible effects
Specific limits	:	

Classified	:	as in Directive 67/548/EEC
Class of danger	:	harmful
R-Phrases	:	(21/22) Harmful in contact with skin and if swallowed
Specific limits	:	

Classified	:	as in Directive 67/548/EEC
Class of danger	:	irritating
R-Phrases	:	(36/37) Irritating to eyes and respiratory system
Specific limits	:	

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use	:	type	
Category	:	Non dispersive use	
Remark	:	Company (site): Degussa AG, Herne (Germany)	
Type of use	:	type	
Category	:	Use in closed system	
Remark	:	Company (site): Degussa AG, Herne (Germany) Company (site): Dow Chemical Company, Institute, WV (USA)	(24) (130)
Type of use	:	type	
Category	:	Wide dispersive use	
Remark	:	Company (site): Dow Chemical Company, Institute, WV (USA)	(130)
Type of use	:	industrial	
Category	:	Agricultural industry	
Remark	:	Company (site): Degussa AG, Herne (Germany) Company (site): Dow Chemical Company, Institute, WV (USA)	(24) (130)
Type of use	:	industrial	
Category	:	Basic industry: basic chemicals	
Remark	:	Company (site): Degussa AG, Herne (Germany) Company (site): Dow Chemical Company, Institute, WV (USA)	(24) (130)
Type of use	:	industrial	
Category	:	Chemical industry: used in synthesis	
Remark	:	Company (site): Degussa AG, Herne (Germany) Company (site): Dow Chemical Company, Institute, WV (USA)	(24) (130)

Type of use : industrial
Category : Paints, lacquers and varnishes industry

Remark : Company (site): Degussa AG, Herne (Germany)
Company (site): Dow Chemical Company, Institute, WV (USA)
(24) (130)

Type of use : use
Category : Intermediates

Remark : Company (site): Degussa AG, Herne (Germany)
Company (site): Dow Chemical Company, Institute, WV (USA)
(24) (130)

Type of use : use
Category : Solvents

Remark : Company (site): Degussa AG, Herne (Germany)
Company (site): Dow Chemical Company, Institute, WV (USA)
(24) (130)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

Origin of substance :
Type : Production

Method : Continuous process; water is a by-product
Remark : Company (site): Daicel Chemical Industries Ltd., Aboshi, Himeji (Japan)
Company (site): Degussa AG, Herne (Germany)
Company (site): Dow Chemical Company, Institute, WV (USA)
(20) (24) (130)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : MAK (DE)
Limit value : 11 mg/m3

Country : Germany
Flag : Critical study for SIDS endpoint
(25)

Type of limit : MAK (DE)
Limit value : 2 ml/m3

Country : Germany
(25)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : KBwS (DE)
Labelled by : KBwS (DE)
Class of danger : 1 (weakly water polluting)

Country : Germany
Remark : No. 1183 in catalogue

(25)

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Stoerfallverordnung (DE)
Substance listed : no
No. in Seveso directive :

Country : Germany
Remark : Stoerfallverordnung 2000

(25)

1.8.5 AIR POLLUTION

Classified by : TA-Luft (DE)
Labelled by : TA-Luft (DE)
Number : other: 5.2.5, 5.2.5E
Class of danger : I

Country : Germany

(25)

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : Human: exposure by production
Exposure to the : Substance

Remark : Occupational exposure data
Result : Median workplace exposure < 0.5 ppm (2,87 mg/m3; 15 min)

(3)

Source of exposure : Human: exposure by production

Exposure to the : Substance

Remark : Company (site): Degussa AG, Herne (Germany)
Occupational exposure data

Result : Range of measurements: not reported
Median: << 1/5 of MAK (< 2.2 mg/m3)
95%-percentil: not reported

Test condition : Years of measurements: annual
Number of samples: not reported
Method: "state of the art" / gas chromatography
Sampling Period: 8 hour shift

(24)

Source of exposure : Human: exposure by production
Exposure to the : Substance

Remark : Company (site): Dow Chemical Company, Institute, WV (USA)
Occupational exposure data

Result : Range of measurements: 0.06-4.07 ppm (0.34-23.4 mg/m3)
(S. Chas: 1.3-2.4 ppm = 7.5-13.8 mg/m3)
Median: 1.95 ppm = 11.2 mg/m3
(S. Chas: 1.33 ppm = 7.6 mg/m3)
95%-percentil: 3.63 ppm = 20.9 mg/m3
(S. Chas: 2.18 ppm = 12.5 mg/m3)

Test condition : Years of measurements: 1995-1999
Number of samples: 68 (+ 5 at S. Chas. Tech Center)
Method: Personnel Monitoring and Area / Source Sampling
Sampling Period: 15 min STEL or long term up to 8 hours

(130)

Source of exposure : Environment: exposure from production
Exposure to the : Substance

Remark : Release to environment (production site)

Result : The concentration of isophorone in the receiving river (Arc) is estimated to be well below 1 ppm (1 mg/l).

(3)

Source of exposure : Environment: exposure from production
Exposure to the : Substance

Remark : Company (site): Daicel Chemical Industries Ltd., Aboshi, Himeji (Japan)
Release to environment (production site)

Result : Emission to air in year
- not reported
Release to waste water in year
- to WWTP: max. 80 t/year
calculated as: ca. 6300 m3/year x 12 g/l (solubility)
Monitoring in WWTP effluent
- no data available
Waste
- no data available

(20)

Source of exposure : Environment: exposure from production
Exposure to the : Substance

Remark : Company (site): Degussa AG, Herne (Germany)
Release to environment (production site)

Result : Emission to air in year
- Total combustion of offgas in a thermal oxidizer
Release to waste water in year
- < 1 mg/l x 16,000 m3/year = < 16 kg/year, to WWTP;
Monitoring in WWTP effluent
- no data available
Waste
- No solid solid waste
- Total combustion of liquid waste (5 t/h) in thermal oxidizer

(24)

Source of exposure : Environment: exposure from production
Exposure to the : Substance

Remark : Company (site): Dow Chemical Company, Institute, WV (USA)
Release to environment (production site)

Result : Emission to air in year
- 1999: 468 kg/year
- 2000: 310 kg/year
- 2001: 1221 kg/year
Release to waste water in year
- 1999: 1080 kg/year
- 2000: 1027 kg/year
- 2001: 792 kg/year
Monitoring in WWTP effluent
- 1999-2001 (36 samples): 42 ppb (mg/m3) average
flow rate 0.17 m3/s
300 days/year x 0.17 m3/s x 42 mg/m3 x 3600x24 s/day x 1 kg / 1 000 000 mg = annual load ca. 185 kg
WWTP sludge to landfill

(130)

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value : = -8.1 °C
Decomposition : no, at °C
Sublimation : no
Method : other: not specified
Year :
GLP : no data
Test substance : no data

Remark : Probably identical with other value but with precision reported higher by one digit.
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint

(4) (9) (59) (96)

Value : = -8 °C
Sublimation : no
Method : other: not specified
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(52) (115) (125) (143)

2.2 BOILING POINT

Value : = 215.3 °C at 1013 hPa
Decomposition :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Remark : All values are essentially identical. The selected value is accompanied by the most detailed additional information.
Result : The azeotrope with water boils at 99.5 degree C and has an isophorone concentration of 16.1 wt.%
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint

(9)

Value : = 215 °C at 1013 hPa
Decomposition :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(52) (59) (143)

Value : = 215.2 °C at 1013 hPa
Decomposition :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(4) (96)

Value : = 215.5 °C at 1013 hPa
Decomposition :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(115)

2.3 DENSITY

Type : density
Value : = .9215 g/cm³ at 20 °C
Method :
Year :
GLP : no data
Test substance : no data

Remark : From a set of essentially identical values with similar validity, the one with the highest number of digits reported and closest to the mean is selected.
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
(4)

Type : density
Value : = .92 g/cm³ at 20 °C
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(52) (59) (143)

Type : density
Value : = .921 g/cm³ at 20 °C
Method : other: no data
Year :
GLP : no data
Test substance :

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(4)

Type : density
Value : = .922 g/cm³ at 20 °C
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(102) (115)

Type : density
Value : = .9229 g/cm³ at 20 °C
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(9)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .4 hPa at 20 °C
Decomposition : no
Method : other (measured): no data
Year :
GLP : no
Test substance : no data

Remark : The selected value is supported by a vapour pressure curve.
Result : Values at higher temperatures:
---
50 degree C: 2.7 hPa
100 degree C: 30 hPa
150 degree C: 180 hPa
200 degree C: 710 hPa
250 degree C: 2200 hPa
300 degree C: 5300 hPa
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint

(59)

Value : = .33 hPa at 20 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions

Data from handbook or collection of data

(4) (52)

Value : = .506 hPa at 20 °C
Decomposition : no
Method : other (measured)
Year :
GLP : no data
Test substance : no data

Remark : Reported by Verschueren as 0.38 mm Hg at 20 °C
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(125) (143)

Value : = 2.7 hPa at 50 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : no data

Remark : Value at 30 °C: 0.73 hPa
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(4)

Value : = .59 hPa at 25 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : no data

Remark : reported as 0.44 mm Hg
Reliability : (4) not assignable
Documentation insufficient for assessment

(140)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 1.67 at 20 °C
pH value :
Method : other (measured): Flask-shaking Method
Year : 1964
GLP : no
Test substance : no data

Remark : As the HPLC method is an indirect method, the value obtained by the Flask-shaking method is preferred among the two values with highest validity.
Test condition : multiply distilled octanol saturated with distilled water;
distilled water saturated with multiply distilled octanol;
shaking for 15 minutes, further 30 minutes at room temperature;
separation of phases and centrifugation;
ca. 1 mg/l solution in one solvent;
equilibration with other solvent at 20 +/- 1 degree C;

centrifugation at 20 +/- 1 degree C with 27,000 g for 30 min;
chemical analysis

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(142)

Partition coefficient : octanol-water
Log pow : = 1.73 at °C
pH value :
Method : other (measured): HPLC Method
Year : 1980
GLP : no data
Test substance : no data

Test condition : reverse-phase HPLC column 3.9 mm x 30 cm;
eluent methanol / water, initially 40:60,
linear increase to 100:0;
flow rate 2.0 ml/min;
six reference substances for calibration curve

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

(142)

Partition coefficient :
Log pow : = 1.7 at °C
pH value :
Method :
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data

(125)

Partition coefficient :
Log pow : = 1.66 at 23 °C
pH value :
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 1981
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Documentation insufficient for assessment

(62)

Partition coefficient :
Log pow : = 2.22 at °C
pH value :
Method : other (calculated)
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment

(140)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : = 14.5 g/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : very soluble (> 10000 mg/L)
Stable :
Deg. product :
Method : other: see Test Conditions
Year :
GLP : no data
Test substance : no data

Remark : A significant effect of the substance on pH is not expected from its structure.
Though 12 g/l is reported in most publications, the selected value is preferred because of its better documentation.
Test condition : distilled water passed through XAD-2 resin column for removal of organic impurities;
excess of chemical added to approximately 10 ml;
magnetical stirring in constant-temperature water bath (temperature not reported);
aliquots centrifuged and analyzed until no more change in concentration
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint

(142)

Solubility in : Water
Value : = 12 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : very soluble (> 10000 mg/L)
Stable :
Deg. product :
Method :
Year :
GLP : no data
Test substance : no data

Remark : infinitely miscible with hydrocarbons, alcohols, ethers, esters, ketones and halogenated hydrocarbons;
reported as 1.2 % by C.E.R.I.
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(52) (96) (115) (125) (143)

Solubility in	:	Water
Value	:	= 12 g/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	Apparently the values of solubility in water and solubility of water in have been interchanged in the publication of Braithwaite. Reported as 1.2 wt %
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

(9) (102)

Solubility in	:	Water
Value	:	at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Remark	:	Apparently the values of solubility in water and solubility of water in have been interchanged in the publication.
Result	:	Solubility of water in isophorone: 4.3 % w/w
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

(9) (102)

2.6.2 SURFACE TENSION

Test type	:	
Value	:	= 32 mN/m at 20 °C
Concentration	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	probably value for neat liquid
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

(9)

2.7 FLASH POINT

Value : = 85 °C
Type : closed cup
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(9)

Value : = 95 °C
Type :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(115)

Value : = 96 °C
Type : closed cup
Method : other: ASTM D 3278
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(102)

Value : = 96 °C
Type : open cup
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(52) (59)

Value : = 104 °C
Type : open cup
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(9)

2.8 AUTO FLAMMABILITY

Value : = 460 °C at
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(52) (59)

Value : = 470 °C at
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(115)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

Value : = 2.6 - mPa s (dynamic) at 20 °C
Result :
Method : other: no data
Year :
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
(9) (59)

2.14 ADDITIONAL REMARKS

Memo : Odor threshold in water

Method : Deutsches Einheits-Verfahren zur Wasseruntersuchung B 1/2
"Bestimmung der Geruchsschwellenkonzentration"
Result : odor threshold = 27 mg/l

Concentration: odor recognized
8 mg/l: 2; 12 mg/l: 4; 16 mg/l: 5; 20 mg/l: 5; 28 mg/l: 11;
41 mg/l: 17; 53 mg/l: 18; 81 mg/l: 20 persons

Test condition : - Temperature: 22 degree C
- Equipment: Glass vessels rinsed with methanol, then with oder-free tap water
- concentration series in odor-free tap water:
  - screening test for range-finding:
    0.08 / 0.81 / 8.1 / 81 / 810 / 8100 mg/l
  - main test with closer concentrations:
    8 / 12 / 16 / 20 / 28 / 41 / 53 / 81 mg/l
- odor tested by 20 test persons
- overall result = geometric mean of individual results

Reliability : (1) valid without restriction
Test procedure in accordance with national standard methods (147)

Memo : Odor

Result : camphor-like
Reliability : (2) valid with restrictions
Data from handbook or collection of data (115) (116)

Memo : Odor threshold in air

Remark : Value from literature search. Original reference not reported.
Result : Air odor threshold: 0.20 ppm (v/v) = 1.15 mg/m3
Reliability : (2) valid with restrictions
Data from handbook or collection of data (1)

Memo : Odor

Result : peppermint-like
Reliability : (4) not assignable
Documentation insufficient for assessment (138)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	other: UV
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (measured)
Year	:	2002
GLP	:	no
Test substance	:	no data
Method	:	Kinetics of the reaction with OH-radicals under simulated atmospheric conditions
Result	:	Rate constant: kOH = (2.4 +- 0.7) E-11 cm3 molec-1 s-1 Assuming a concentration of 500000 OH-radicals/cm3, a half-life of 16 h is calculated
Test condition	:	TEST TYPE: - Test medium: synthetic air - Test system: Smog chamber - Concentration of test substance: 2 - 8 ppm - Generation of sensitizer: methyl nitrite - Pressure: atmospheric - Temperature: 298 K DURATION: 60-75 min REFERENCE SUBSTANCE: cyclohexene
Reliability	:	(2) valid with restrictions Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(89)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	O3
Conc. of sensitizer	:	
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (calculated): Graphical Exposure Modelling System (GEMS)
Year	:	1995
GLP	:	
Test substance	:	
Result	:	Rate constant: kO3 = 5 E-16 cm3 molec-1 s-1 at 25 degrees C Assuming a concentration of 1 E12 O3-molecules/cm3, a half-life of 23 min is calculated
Reliability	:	(2) valid with restrictions Secondary literature; Reer-reviewed dossier
Flag	:	Critical study for SIDS endpoint

(146)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	= .0000000000815 cm³/(molecule*sec)
Degradation	:	= 50 % after .2 day(s)
Deg. product	:	
Method	:	other (calculated): AOP Computer Program, Vers. 1.53, Syracuse Research Center (based on Reference)
Year	:	1995
GLP	:	
Test substance	:	
Reliability	:	(2) valid with restrictions Accepted calculation method

(2)

Type	:	water
Light source	:	other: Low pressure mercury vapor lamp
Light spectrum	:	ca. 254 nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	62 mg/l at °C
INDIRECT PHOTOLYSIS		
Sensitizer	:	other: hydrogen peroxide
Conc. of sensitizer	:	250 mg/l
Rate constant	:	cm³/(molecule*sec)
Degradation	:	> 99.9 % after 60 minute(s)
Deg. product	:	
Method	:	other (measured): see ME freetext
Year	:	1987
GLP	:	no data
Test substance	:	no data
Method	:	UV irradiation induced decomposition of hydrogen peroxide followed by reaction of the resulting OH radicals with the test substance
Remark	:	Because of UV light and high H2O2 concentration not relevant for environmental conditions
Result	:	No degradation was observed in controls with H2O2 or UV irradiation alone, respectively. The rate constant varied with intensity of UV radiation. It was first order with respect to H2O2 concentration and zero order with respect to isophorone concentration.
Test condition	:	intensity of UV radiation: ca. 1210 uW/cm2
Reliability	:	(2) valid with restrictions No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

(8)

Type	:	other
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	
Year	:	

GLP	:	no
Test substance	:	no data
Result	:	lambda(max) at 312 nm (epsilon = 45 M-1 cm-1). It was reported that photolysis of isophorone in water at wavelengths >200 nm gave dimerization products. Dimerization products were also found when isophorone was irradiated at >300 nm in organic solvents. Such dimerization products are unlikely in the aquatic environment under the highly dilute environmental conditions. It was also reported that photolysis of isophorone at >300 nm in air-saturated carbon tetrachloride solution resulted in the loss of isophorone. This information indicates that loss of isophorone could occur via interactions of isophorone with organic substances in aquatic systems.
Reliability	:	(4) not assignable Secondary literature

(15)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Result	:	Atmospheric lifetime < 1 day
Reliability	:	(4) not assignable Insufficient documentation, origin of the result unclear

(75) (77)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Remark	:	There are no appropriate functional groups and no experimental observations.
Result	:	Hydrolysis is not an important process in the aquatic fate of isophorone.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(15)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement	:	concentration at contaminated site
Media	:	air
Concentration	:	<= .132 mg/l
Method	:	see Test Conditions
Country	:	USA: screen printing plant, before 1982
Remark	:	The concentration of isophorone in the solvent mixtures employed was up to 75 %. Only the automatic dryer was vented

to outdoor atmosphere.

Result : time weighted average concentrations of airborne 3,5,5-trimethylcyclohex-2-enone (mg/m3):
(a) at the breathing zone of workers in various jobs;
(b) in the atmosphere of workplaces:

	(a)	n	(b)	n
printing press	132 +/- 31	18	92 +/- 28	10
automatic dryer	55 +/- 19	19	72 +/- 24	8
manual drying	86 +/- 24	15	83 +/- 36	6
paint mixing	102 +/- 32	12	43 +/- 28	6
screen wash	48 +/- 32	14	41 +/- 22	10
general air		20 +/- 10	6	

Test condition : n samples were taken during the active portion of a worker's shift in a screen printing plant on jobs with aparent higher risk of exposure to solvent vapors.
- sampling material: charcoal tubes
- sampling rate: 0.19 l/min
- sampling time: 50 - 90 min
- desorption with carbon disulfide at 25 degree C (1 hour)
- analysis by gas chromatography according to NIOSH recommendations

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

(112)

Type of measurement : concentration at contaminated site
Media : air
Concentration : <= .0015 mg/l
Method : see Test Conditions

Country : Germany: screen printing
Result : The following concentrations of isophorone were found (mg/m3):

Operation	samples*	minimum	median	maximum
Screen cleaning:	1 (13)	0.5	0.5	0.5
Storage:	0 (4)	-	-	-
Manual printing:	4 (4)	0.5	0.7	0.9
Semiautomatic p.:	7 (22)	0.8	1.0	1.5
3/4 automatic p.:	16 (35)	0.4	0.7	1.1
automatic printing:	2 (9)	1.0	1.1	1.1

* number of samples with positive identification of isophorone (total number of samples)

Test condition : Mean concentrations of various solvents in the workplace atmosphere of 11 plants of the screen printing industry were determined (39 workplaces, 87 determinations).
Initially, places of maximum exposure were determined by flame ionization detector (FID).
Samplers were equipped with charcoal (==> solvent elution) or Tenax GR (==> thermodesorption).
Samples were separated by capillary gas chromatography, identified with mass spectrometry / spectra library / authentic samples, and quantified by internal standard.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(5)

Type of measurement : background concentration
Media : surface water
Concentration :
Method : see Test Conditions

Country : Spain: Llobregat River
Result : Isophorone was identified but not quantified in > 75 % of raw water samples from Llobregat River and drinking water prepared thereof.
Test condition : Adsorption to Granular Activated Carbon (CAC), Soxhlet extraction, high-resolution gas chromatography / low resolution mass spectrometry
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(104)

Type of measurement : background concentration
Media : surface water
Concentration : < .1 - .7 µg/l
Method : adsorption on XAD resin at pH 7, elution, GC/MS

Country : The Netherlands: river Lek (near Hagestein)
Result : mean: 0.1 ug/l (maximum: 0.7 ug/l, median < 0.1 ug/l)
Test condition : 50 samples were taken in 1986; detection limit and other details not reported.
Reliability : (4) not assignable
Documentation insufficient for assessment

(90)

Type of measurement : background concentration
Media : surface water
Concentration : < 10 µg/l
Method : other: U.S. EPA monitoring programme

Country : USA
Remark : U.S. EPA STORET database
Result : In 795 water samples, isophorone was identified in 1.0 % at a median concentration of < 10 ug/l
Test condition : Database search Oct. 1983 - Jan 1984
total concentration in unfiltered water
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(122)

Type of measurement : background concentration
Media : surface water
Concentration : = 10 µg/l
Method : no data

Country : USA: Washington D.C.
Result : 10 ug/l was detected in urban runoff of Washington, D.C.; the detection frequency was 4 % among 86 samples
Test condition : Results from Nationwide Urban Runoff Program (NURP) as received by 31 July 1982; 15 cities reporting.
Information on analytical procedures is not reported.
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(19)

Type of measurement : background concentration
Media : surface water
Concentration : < .01 µg/l
Method : see Test Conditions

Country : USA: Delaware river
Remark : Over 120 major chemical manufacturing plants are located along this river. It provides 50 % of Philadelphia's water.
Result : August 1976: not detected
March 1977: "trace"
Test condition : August 1976: 3.5 l samples from 11 locations between river miles 78 and 132
March 1977: 21 l samples from 5 locations (same range)
Analysis usually within 24 hours: Extraction with methylene chloride, liquid chromatographic cleanup, gas chromatography / mass spectrometry identification and quantitation
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(113) (138)

Type of measurement : concentration at contaminated site
Media : surface water
Concentration : ca. 40 µg/l
Method : GC / MS / computer

Country : USA (location not reported)
Result : isophorone was determined in the waste waters of one of the two plants at a level of 0.04 mg/l +/- 30 %
Test condition : wastewater from two tire manufacturing plants was sampled, extracted with dichloromethane, dried and analyzed by gas chromatography / mass spectrometry / computer system
Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

(74)

Type of measurement : concentration at contaminated site
Media : surface water
Concentration : <= .1 mg/l
Method : see Test Conditions

Country : USA: Delaware river
Result : Isophorone concentrations determined:
Plant effluent: unresolved
Philadelphia NE treatment plant influent: 100 ug/l
Philadelphia NE treatment plant effluent: 10 ug/l
Two miles downstream 3 ug/l
Further 2 miles downstream 0.6 ug/l
Torresdale water treatment plant influent: traces
(another 2 miles downstream)
Another 8 miles downstream: not detected
Torresdale water treatment plant effluent: traces
Test condition : August 1977: samples of varying volumes from 8 locations between the effluent of a chemical plant and Philadelphia's main drinking water supply
Analysis usually within 24 hours: Extraction with methylene chloride, liquid chromatographic cleanup, gas chromatography / mass spectrometry identification and quantitation

Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment (114)
Type of measurement	:	concentration at contaminated site
Media	:	surface water
Concentration	:	< 10 µg/l
Method	:	other: U.S. EPA monitoring programme
Country	:	USA
Remark	:	U.S. EPA STORET database
Result	:	In 1,272 industrial effluent samples, isophorone was identified in 1.6 % at a median concentration of < 10 ug/l
Test condition	:	Database search Oct. 1983 - Jan 1984 total concentration in unfiltered water
Reliability	:	(2) valid with restrictions Data from handbook or collection of data (122)
Type of measurement	:	background concentration
Media	:	drinking water
Concentration	:	<= .02 µg/l
Method	:	no data
Country	:	USA
Result	:	Cincinnati (OH): 0.02 ug/l Miami (FL): not detected Seattle (WA): not detected Ottumwa (IA): not detected Philadelphia (PA): not detected Tucson (AR): not detected New York (NY): not detected Lawrence (MA): not detected Grand Forks (ND): not detected Terrebonne Parish (LA): not detected
Reliability	:	(4) not assignable Documentation insufficient for assessment (138) (139)
Type of measurement	:	background concentration
Media	:	drinking water
Concentration	:	<= 1.5 - 2.9 µg/l
Method	:	no data
Country	:	USA: Louisiana / New Orleans area
Result	:	Highest measured isophorone concentrations in finished drinking water from New Orleans area: Carrolton Water Plant (New Orleans, LA): 1.5 ug/l Two water treatment sites in Jefferson Parish: 2.2; 2.9 ug/l
Reliability	:	(4) not assignable Documentation insufficient for assessment (138) (139)
Type of measurement	:	concentration at contaminated site
Media	:	drinking water
Concentration	:	<= 9.5 µg/l
Method	:	no data
Country	:	USA (location not reported)

Test condition	:	Maximum value found in extensive literature search
Reliability	:	(4) not assignable Documentation insufficient for assessment (138) (139)
Type of measurement	:	concentration at contaminated site
Media	:	ground water
Concentration	:	<= 10 µg/l
Method	:	no data
Country	:	The Netherlands
Reliability	:	(2) valid with restrictions Data from handbook or collection of data (151)
Type of measurement	:	concentration at contaminated site
Media	:	other: wastewater
Concentration	:	<= 5.8 mg/l
Method	:	purge & trap
Country	:	USA: Oil shale reserves in western Colorado and Utah / shale oil facilities
Remark	:	The results indicate natural occurrence in shale oil.
Result	:	Concentration of isophorone in wastewater from various processes in shale oil refinery ranged from 0.34 to 5.8 mg/l. The substance was not detected in the headspace above the wastewater and in air samples from oil shale wastewater facility, urban, and rural origin.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment (50)
Type of measurement	:	background concentration
Media	:	sediment
Concentration	:	<= 2.995 mg/kg soil dw
Method	:	see Test Conditions
Country	:	Canada: North Saskatchewan River, 960 km reach, 9 sampling sites
Remark	:	Concentrations probably refer to wet weight.
Result	:	suspended sediments: detected at 1 out of 9 sites (Borden Bridge): 355 ug/kg; bottom sediments: detected at 1 out of 9 sites (Battlefords): 2995 ug/kg; water: not detected at 9 sites
Test condition	:	Samples were taken in August 1983 during a stable low flow period: Suspended sediments: river water was taken from a depth of 0.3 m and the silt-clay fraction (sediment particles < 0.62 um) was obtained by centrifugation; Bottom sediments: predominantly sand or larger particles; Water: 4 l samples were collected from a depth of 20 cm Analysis: Sediment samples were Soxhlet extracted with 1:1 (v/v) acetone / hexane, preconcentrated, extracted from aqueous NaOH with dichloromethane, preconcentrated, fractionated by elution over silica gel with hexane / dichloromethane at various ratios. Water samples were extracted with dichloromethane, dried and

preconcentrated.
All samples were analyzed by GC/MS/data base.
Fortified samples and methods blanks were analyzed for quality assurance. Recoveries and detection limits are not reported in the publication.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(100)

Type of measurement : background concentration
Media : sediment
Concentration : = .0009 - .012 mg/kg soil dw
Method : see Method freetext

Country : USA: Passes between Lake Pontchartrain and Gulf of Mexico / Louisiana
Method : extraction with diethyl ether,
gel permeation with dichloromethane,
gas chromatography / mass spectrometry.
Use of recovery standards, internal standards, concentration steps.
Recovery and detection limit not reported.
Result : Inner harbor navigation channel: 0.9 ng/g (mean of 8 samples)
The Rigolets: 10 ng/g (1 sample)
Chef Menteur Pass: 12 ng/g (1 sample)
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(86)

Type of measurement : background concentration
Media : sediment
Concentration : < .5 mg/kg soil dw
Method : other: U.S. EPA monitoring programme

Country : USA
Remark : U.S. EPA STORET database
Result : In 318 sediment samples, isophorone was identified in 0.0 % at a median concentration of < 500 ug/kg dry weight.
Test condition : Database search Oct. 1983 - Jan 1984
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(122)

Type of measurement : background concentration
Media : biota
Concentration : <= .038
Method : see Method freetext

Country : USA: Passes between Lake Pontchartrain and Gulf of Mexico / Louisiana
Method : extraction with diethyl ether,
gel permeation with dichloromethane,
gas chromatography / mass spectrometry.
Use of recovery standards, internal standards, concentration steps.
Recovery and detection limit not reported.
Result : Inner harbor navigation channel: 38 ng/g wet weight (mean of

8 oyster samples)
The Rigolets: not detected (1 composite clam sample)
Chef Menteur Pass: not detected (1 composite clam sample)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(86)

Type of measurement : background concentration
Media : biota
Concentration : < 2.5
Method : other: U.S. EPA monitoring programme

Country : USA
Remark : U.S. EPA STORET database
Result : In 123 aquatic biota samples, isophorone was identified in 0 % at a median concentration of < 2.5 mg/kg wet weight
Test condition : Database search Oct. 1983 - Jan 1984
Reliability : (2) valid with restrictions
Data from handbook or collection of data

(122)

Type of measurement : concentration at contaminated site
Media : biota
Concentration :
Method : see Test Conditions

Country : USA: Michigan
Result : range of mean concentrations in resident nearshore fish collected from 13 Lake Michigan tributaries and Grand Traverse Bay (Michigan, USA):

	mg/kg wet weight
Ambloplites rupestris:	< 0.2 - 1.44
Amia calva:	< 0.2 - 0.76
Cyprinus sp. (Fish, fresh water):	< 0.2 - 3.13
Esox lucius (Fish, fresh water):	< 0.2 - 0.48
Ictalurus punctatus (Fish, fresh water):	< 0.2
Lepomis gibbosus (Fish, fresh water):	0.4
Micropterus dolomieui (Fish, fresh water, marine)	0.74 - 3.61
Micropterus salmoides (Fish, fresh water):	0.72
Salvelinus namaycush (Fish, fresh water):	2.33

Test condition : 15 samples of two fish species were collected at each sampling site during fall 1983.
whole body fish samples were homogenized and extracted by Soxhlet methods according to USEPA, Method 625, Federal Register 44.223 (1979); GPC cleanup and GC/MS analysis followed. Detection limit: 0.2 mg/kg wet weight of fish
Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

(16)

Type of measurement : other: toys
Media : other: PVC toys
Concentration : <= 4.214
Method : see Test Conditions

Country : Belgium: Toys from Asia on the market
Remark : The toys investigated were for small children who put

everything in their mouths. The presence of isophorone in such toys means that they are not in compliance with Belgian legislation, though they were on the Belgian market.

Result : Isophorone was detected in 6 out of 7 PVC plastic toys from Asia at levels between 98 and 4214 mg/kg.
Migration to water was quantified for 5 of these samples and was between 0.1 and 2.4 mg/dm2.

Test condition : - Identification: Dissolution in diethyl ether, gas chromatography with internal standards.
- Quantification: Dissolution in methanol, gas chromatography with internal standard, calculation of concentration from peak areas of test solution and standard solutions.
- Migration: ca. 2 dm2 in contact with ca. 100 ml water at 37 degree C for 24 hours, adsorption to SEPAK C18 cartridge, elution with acetonitrile, addition of internal standard, quantification.
- Recovery was determined to be > 90 %

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(71)

Type of measurement : other: toys
Media : other: toys, mostly PVC
Concentration :
Method : see Test Conditions

Country : 17 countries of purchase, >= 14 countries of manufacture
Result : Isophorone was identified. Frequency and concentrations are not reported.
Test condition : 72 toys purchased in 17 countries,
reduction to small pieces,
elution with hexane (sonication),
gas chromatography,
mass spectrometry / spectra match with expert check
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(124)

Type of measurement : other: toys
Media : other: PVC toys, printed or painted
Concentration :
Method : migration, HPLC; see Test Conditions

Country : Germany: various toys of unreported origin, probably from German market
Result : Results are reported for 7 out of approximately 150 toys:
Booklet A: 1.2 mg/dm2
Booklet B: not detected
PVC doll: not detected
PVC figure MM: not detected
inflatable figure: 0.2 mg/dm2
inflatable pyramid: 3.3 mg/dm2
PVC figure: not detected
Test condition : - toy sample sealed in foil with 100 ml demineralized water;
- 24 hours at 40 degree C;
- subsequent treatment of foil with 1:1 methanol / tetryhydrofuran for 10 min at 40 degree C;
- HPLC analysis of combined solvents, gradient elution

- UV detection at 280 nm
- control: foil
- recovery and sensitivity tested with standard solution: recovery ca. 95 %, detection limit 50 ng (50 ng/100 ml = 0.5 ug/l; 50 ng/1 dm2 = 50 ng/dm2)

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

(42)

Type of measurement : background concentration
Media : other: fly ash from coal power plant
Concentration : <= .49
Method : see Test Conditions

Country : USA: New Mexico
Remark : Differences between results for two processes may be due to differences in collection effiencies for specific fractions or to analytical procedures.
Result : Concentration determined in fly ash from
- electrostatic precipitator: 0.49 mg/kg (490 ppb)
- wet scrubber: not detected

Test condition : Fly ash collected at Four Corners Power Station, New Mexico, on 12 Dec. 1979
Conclusion : Probable origin is natural occurrence in the fired coal.
Reliability : (4) not assignable
Documentation insufficient for assessment

(47)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 1995

Result : Volatilization half-life in a model river (flow 1 m/sec) was calculated to 7.5 days, based on a water solubility or 12 g/l and a vapour pressure of 40 Pa at 20 degrees C (Henry's law constant: 0.59 Pa m3/mol).
Based on a water solubility of 17.5 g/l, the volatilization half-life would be 11 days
Reliability : (2) valid with restrictions
Data from peer-reviewed report

(146)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water

Method : Calculation according Mackay, Level I
Year : 2002

Result : Calculated distribution between environmental compartments:
Air: 11.75%
Water: 87.62%
Soil: 0.32%
Bottom Sediment: 0.30%
Suspended Sediment: 0.0005%
Biota: 0.0002%

Test condition : Data used in calculation:
Temperature (°C): 20
Molar mass (g/mol): 138.21
Vapour pressure (Pa): 40
Water solubility (g/l): 14.5
log Kow: 1.67

Volumes in unit world (m3):
Air: 6 000 000 000
Water: 7 000 000
Soil: 45 000
Sediment: 21 000
Susp. sediment: 35
Biota (fish): 7

Reliability : (2) valid with restrictions
Generally accepted calculation method
Flag : Critical study for SIDS endpoint

(141)

Media : water - air
Method : other (calculation)
Year : 2001

Method : vapour pressure x molecular weight / water solubility
Result : Henry's law constant at 20 degree C:
40 Pa * 138.21 g/mol / 14,500 g/m3 = 0.38 Pa m3/mol
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
27.06.2003

(23)

Media : water - air
Method : other (calculation)
Year : 1988

Result : Henry's law constant at 20 degree C: 0.58 Pa m3/mol
calculation based on water solubility (12 g/l) and vapor pressure (50 Pa)
Reliability : (2) valid with restrictions
Generally accepted calculation method

(125)

Media : water - air
Method : other (calculation): according to Mackay and Shiu
Year : 1981

Remark : K = vapour liquid equilibrium constant of substance in infinitely diluted aqueous solution; dimensionless Henry's law constant in terms of mole fractions
Result : K = 17 at 100 degree C; K = 0.32 at 25 degree C

Reliability	:	(2) valid with restrictions Generally accepted calculation method (70)
Media	:	water - air
Method	:	other (calculation): distribution ratio = solubility (ppm w/v)/volatility (ppm v/v) x 24400/molecular weight
Year	:	1978
Method	:	Calculation based on literature search: water solubility (12.000 g/ml) x 24.400 ml/mole / vapour pressure (450 ppm [= 0.34 mm Hg = 0.46 hPa]) / molecular weight (138 g/mole) = ca. 4800/ppm
Remark	:	The distribution ratio is equivalent to the reciprocal value of the Henry's Law constant.
Result	:	distribution ratio (w/v) = 4800 at 25 degree C
Reliability	:	(2) valid with restrictions Data from handbook or collection of data (1)
Media	:	other: vapour - particle bound
Method	:	
Year	:	
Result	:	In the atmosphere, isophorone should exist primarily in the vapor phase.
Reliability	:	(4) not assignable Documentation insufficient for assessment (140)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Concentration	:	10 mg/l related to DOC (Dissolved Organic Carbon) related to
Contact time	:	
Degradation	:	= 95 (±) % after 28 day(s)
Result	:	readily biodegradable
Kinetic of testsubst.	:	7 day(s) = 5 % 14 day(s) = 71 % 21 day(s) = 93 % 27 day(s) = 99 % 28 day(s) = 95 %
Control substance	:	Benzoic acid, sodium salt
Kinetic	:	14 day(s) = 98 % 28 day(s) = 100 %
Deg. product	:	
Method	:	other: Directive 92/69/EEC, part 2 C.4/A
Year	:	1992
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	INOCULUM/TEST ORGANISM - Source: municipal WWTP Marl-Ost, sampled 12 Aug 1992

- Preparation of inoculum: centrifugation (10 min at 1100 g), discard supernatant, resuspend in mineral medium, centrifugation (as above), resuspension of sludge (4.6 g dry weight/l), keep aerated
- Initial cell concentration: 23 mg/l
TEST SYSTEM
- Culturing apparatus: 2000 ml Erlenmeyer flask covered loosely with aluminum sheet, filled with 900 ml test soln.
- Number of culture flasks per concentration: each 2: test substance (11.48 mg DOC/l) and inoculum; control substance (11.69 mg DOC/l) and inoculum, inoculum only
- Aeration device: shaking for 28 days
METHOD OF PREPARATION OF TEST SOLUTION:
- stock solution: 765 mg DOC/l
DURATION OF THE TEST: 28 days
ANALYTICAL PARAMETER: DOC (Carbon analyzer, Shimadzu), determination with and without removal of inorganic carbon
TEST CONDITIONS
- Composition of medium:
a 8.5 g KH_2PO_4/l
21.75 g K_2HPO_4/l
33.3 g Na_2HPO_4/l
20.0 g $(NH_4)Cl$/l
b 22.5 g $MgSO_4 \times 7 H_2O$/l
c 27.5 g $CaCl_2$/l
d 0.25 g $FeCl_3 \times 6 H_2O$/l
- Test temperature: 21.9-22.0 degree C
REFERENCE SUBSTANCE: sodium benzoate > 99.5 % (Fluka), stock solution 568 mg DOC/l

Test substance : Huels AG, purity > 99.5 % (GC area), produced 30 Jun 1992, ID 3633/81431

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

(63)

Type : aerobic
Inoculum : activated sludge, domestic, non-adapted
Concentration : 10.4 mg/l related to DOC (Dissolved Organic Carbon)
related to
Contact time : 3 hour(s)
Degradation : = 68.9 (±10.9) % after
Result : other
Deg. product :
Method : OECD Guide-line 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Unit Test"
Year : 1997
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The test was performed in Feb/Mar 1984.
Result : The mean of 24 measurements at approximately regular intervals is 68.86 +/- 10.90 % degradation.
Test condition : INOCULUM/TEST ORGANISM
- Source: municipal WWTP Marl-West, sampled 17 Feb 1984
- Pretreatment: fed into test apparatus ca. 40 min after sampling
TEST SYSTEM
- Culturing apparatus: flow-through
- Number of culture flasks per concentration: 1
- Aeration device: pump

- Measuring equipment: TOC 500 Infrared analyzer
- Closed vessels used: no
DURATION OF THE TEST: 33 days
ANALYTICAL PARAMETER: DOC
SAMPLING:
TEST CONDITIONS
- Composition of synthetic waste water:
88 mg/l Pepton (Unipath)
55 mg/l meat extract (Unipath)
15 mg/l urea, CAS RN 57-13-6
3.5 mg/l sodium chloride p.a., CAS RN 7647-14-5
2 mg/l calcium chloride x 2 H2O p.a.
1 mg/l magnesium sulfate x 7 H2O p.a.
14 mg/l K2HPO4 p.a.
- Additional nutrition substrate A:
32 g/l Pepton
22 g/l meat extract
6 g/l urea
1.4 g/l sodium chloride
0.8 g/l calcium chloride x 2 H2O
0.4 g/l magnesium sulfate x 7 H2O
substrate B
- 33.5 g/l K2HPO4 is stored separately
- 47 g/l NaHCO3 is stored separately
- 5 ml K2HPO4 soln. + 25 ml NaHCO3 soln + 11 l tap water
This soln. and nutrition substrate A (30 ml/l) are added separately
mean retention time: 3 hours.

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

(69)

Type : aerobic
Inoculum : activated sludge, domestic, non-adapted
Concentration : 1290 mg/l related to DOC (Dissolved Organic Carbon)
related to
Contact time :
Degradation : = 100 (±) % after 21 day(s)
Result : inherently biodegradable
Kinetic of testsubst. : 3 hour(s) = 3 %
3 day(s) = 5 %
9 day(s) = 86 %
14 day(s) = 89 %
%
Control substance : Diethylene glycol
Kinetic : 3 day(s) = 1 %
9 day(s) = 100 %
Deg. product :
Method : Directive 87/302/EEC, part C, p. 99 "Biodegradation: Zahn-Wellens test"
Year : 1996
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The test was performed in Nov/Dec 1985.
Test condition : INOCULUM/TEST ORGANISM
- Source: municipal WWTP Marl-West
DURATION OF THE TEST: 28 days
ANALYTICAL PARAMETER: DOC
TEST CONDITIONS
- Test temperature: 20-22 degree C

- pH value: 5.8 - 7.3
- Concentration of suspended solids: 1 g inoculum/l (dry wt)

Test substance : Huels AG
Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
(64)

Type : aerobic
Inoculum : other: settled domestic sewage
Concentration : 5 mg/l related to Test substance
10 mg/l related to Test substance
Contact time :
Degradation : = 100 (±) % after 7 day(s)
Result :
Control substance : other: Phenol
Kinetic : %
%
Deg. product :
Method : other: Static-Culture Flask-Screening Procedure
Year : 1967
GLP : no data
Test substance : no data

Remark : minimum sensitivity of the gaschromatographical test substance determination: ca. 0.1 mg/l
Result : Metabolism probably involves the oxidation of the allylic methyl group of isophorone to a carboxylic acid group, which is followed by the rupture of the cyclohexane ring and decarboxylation.
Test condition : TEST SYSTEM
- Culturing apparatus: cotton-stoppered erlenmeyer flask
- Number of culture flasks per concentration: 3
DURATION OF THE TEST: 28 days
ANALYTICAL PARAMETER: TOC; COD; gas chromatography / FID after solvent extraction
SAMPLING: weekly
TEST CONDITIONS
- Additional substrate: 5 mg yeast extract/l
- Test temperature: 25 degree C
- Other relevant factors: test performed in darkness
CONTROLS: inoculum control, substrate control
REFERENCE SUBSTANCE: phenol
Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail
(128)

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time :
Degradation : = 1.5 (±) % after 14 day(s)
Result : other
Deg. product :
Method : other: see remarks
Year : 1992
GLP : no data
Test substance : no data

Remark : The test was conducted in accordance with "Biodegradation

		test of chemical substances by microorganisms etc." stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No.1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test (I)" stipulated in the OECD Guidelines for Testing of Chemicals (1981)
Test condition	:	30 mg/l activated sludge 300 ml test solution at 25 degree C
Reliability	:	(3) invalid High test substance concentration probably toxic for inoculum

(96)

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Concentration	:	100 mg/l related to Test substance related to
Contact time	:	
Degradation	:	= (±) % after
Result	:	other: See Remark and Result freetexts
Control substance	:	Aniline
Kinetic	:	% %
Deg. product	:	
Method	:	other: Respirometric Oxygen Uptake Test
Year	:	1990
GLP	:	no data
Test substance	:	no data
Remark	:	Degradation was determined as BOD related to ThOD (theoretical oxygen demand). Only kinetic data are reported for isophorone, i.e. no data on specific test duration and overall degradation.
Result	:	Duration of lag phase: 22.3 days Initiation of declining growth phase: 23.7 days Termination of declining growth phase: 25.4 days Growth yield: 0.43 mg biomass/mg test substance Maximum growth rate: 1.57 / day
Test condition	:	INOCULUM/TEST ORGANISM - Source: Little Miami wastewater treatment plant, Cincinnati (Ohio) - Pretreatment: aeration for 24 hours - Initial concentration: 30 mg total solids/l TEST SYSTEM - Culturing apparatus: Voith Sapromat B-12 - Number of culture flasks per concentration: 2 - Closed vessels used: yes DURATION OF THE TEST: between 28 to 50 days ANALYTICAL PARAMETER: COD, DOC, specific analysis SAMPLING: continuous monitoring TEST CONDITIONS - Additional substrate: OECD synthetic medium (solutions of mineral salts, trace salts, yeast extract) - Test temperature: 25 degree C - Concentration of suspended solids: 30 mg/l initially CONTROLS: compound control; inoculum control; toxicity control REFERENCE SUBSTANCE: aniline (100 mg/l)
Reliability	:	(3) invalid High test substance concentration probably toxic for

inoculum

(127)

Type : Aerobic
Inoculum : domestic sewage, non-adapted
Concentration : 3 mg/l related to Test substance
10 mg/l related to Test substance
Contact time :
Degradation : = 42 (±) % after 20 day(s)
Result : Other
Kinetic of testsubst. : 5 day(s) = 0 %
10 day(s) = 13 %
15 day(s) = 47 %
20 day(s) = 42 %
%
Deg. product :
Method : other: Standard Method for the Examination of Water and Wastewater, APHA
Year : 1974
GLP : no data
Test substance : no data

Test condition : INOCULUM/TEST ORGANISM
- Type of sludge: settled domestic wastewater
- Preparation of inoculum: filtration through glass wool
TEST SYSTEM
- Number of culture flasks per concentration: 2
- Aeration device: passing to new bottle, moderate shaking, returning to original bottle
- Measuring equipment: commercial dissolved oxygen meter
- inoculum concentration not reported
INITIAL TEST SUBSTANCE CONCENTRATION:
3 / 7 / 10 mg/l (at least two of these concentrations)
DURATION OF THE TEST: 20 days
ANALYTICAL PARAMETER: dissolved oxygen
TEST CONDITIONS
NITRATE/NITRITE MEASUREMENT:
colorimetrically, assumed to be principally nitrite
CONTROLS: inoculum blank
Reliability : (2) valid with restrictions
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

(106)

Type : Aerobic
Inoculum : other: seawater microorganisms and small amounts of settled raw waste water, non-adapted
Concentration : 3 mg/l related to Test substance
10 mg/l related to Test substance
Contact time :
Degradation : = 9 (±) % after 20 day(s)
Result : Other
Kinetic of testsubst. : 5 = 1 %
10 = 6 %
15 = 7 %
%
%
Deg. product :
Method : other: Standard Method for the Examination of Water and Wastewater, APHA

Year	:	1971
GLP	:	no data
Test substance	:	no data
Test condition	:	INOCULUM/TEST ORGANISM - Type of sludge: seawater - Source: Lavaca Bay, Texas - Pretreatment: addition of small amounts of settled raw wastewater about every 3 to 4 days TEST SYSTEM - inoculum concentration not reported - Number of culture flasks per concentration: 2 - Aeration device: passing to new bottle, moderate shaking, returning to original bottle - Measuring equipment: commercial dissolved oxygen meter - Synthetic seawater: 557.37 g NaCl, 27.20 g CaSO4, 63.36 g MgSO4 x 7 H2O, 168.30 g MgCl2, 15.84 g KCl, 3.14 g MgBr2 x 6 H2O, all dissolved in 20 l of distilled water in this order INITIAL TEST SUBSTANCE CONCENTRATION: 3 / 7 / 10 mg/l (at least two of these concentrations) DURATION OF THE TEST: 20 days ANALYTICAL PARAMETER: dissolved oxygen TEST CONDITIONS NITRATE/NITRITE MEASUREMENT: colorimetrically, assumed to be principally nitrite CONTROLS: inoculum blank
Reliability	:	(2) valid with restrictions No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

(106)

Type	:	Aerobic
Inoculum	:	activated sludge, adapted
Concentration	:	100 µg/l related to Test substance related to
Contact time	:	
Degradation	:	ca. 17 - 98 (±) % after
Result	:	
Method	:	four different continuous flow systems (pilot plants)
Result	:	Mean removal in test systems: 17 % in Trickling filter 22 % in Aerated lagoon 25 % in Facultative lagoon 98 % in Activated sludge
Test condition	:	INOCULUM/TEST ORGANISM - Pretreatment: adaptation for 30 days before first sampling INITIAL TEST SUBSTANCE CONCENTRATION: 100 ug/l METHOD OF PREPARATION OF TEST SOLUTION: Secondary effluent, which had been passed through a bed of granular activated carbon, was mixed 1:1 with raw wastewater, spiked with test substance to obtain 100 ug/l, and equilibrated with solids in the wastewater. DURATION OF THE TEST: 8 months ANALYTICAL PARAMETER: test substance concentration; GC/MS SAMPLING: 24 hour composites from grab samples collected six times per day; sampling on 2 or 3 consecutive days at approximately 6-week intervals REFERENCE SUBSTANCE: isotope labelled identical substances

were added to samples as internal standards

Reliability : (3) invalid
Unsuitable test system

(46)

Type : Aerobic
Inoculum :
Deg. product :
Method : other: MITI test
Year :
GLP : no data
Test substance : no data

Result : Isophorone is classified as "degradation-resistant".
Test condition : no data available
Reliability : (4) not assignable
Secondary literature

(76)

Method : Analytical determination of removal degree in treatment facilities
Result : Activated sludge treatment (industrial): >0% removal, influent concentration 10 µg/l
Aerated lagoon treatment (industrial): 33% removal, influent concentration 3 µg/l
Reliability : (4) not assignable
Secondary literature

(103)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 14 day(s) at 16 °C
Concentration : 92.4 µg/l
BCF : = 7
Elimination : Yes
Method : other: Flow-through Bioaccumulation Test
Year : 1980
GLP : no data
Test substance : other TS: 14C-labelled

Remark : BCF is related to whole fish; elimination half-life in tissues: 1 d
Result : Half-life in tissues = 1 day
Test condition : TEST ORGANISM
- bluegill sunfish (Lepomis macrochirus)
- source: commercial fish farm in Connecticut
- wet weight 0.37 +/- 0.18 to 0.94 +/- 0.34 g;
- length 25 +/- 3 to 32 +/- 4 mm;
- acclimation period 30 days

TEST EQUIPMENT
- well water, pH 7.1, total hardness: 35 mg/l as CaCO3;
- glass aquaria of 40 cm length, 20 cm width, 25 cm height / 19 cm depth;

- turnover rate six to seven aquarium volumes per day;
- temperature 16 +/- 1 degree C;
- no aeration; regular determination of dissolved oxygen;
TEST PROCEDURE
- 100 fish per aquarium;
- 50 ul (out of 50 ml) of stock solution thoroughly mixed with 500 ml water before introduction into aquarium;
- dry pelleted food ad libitum on alternating days;
- removal of feces as deemed necessary;
- duration until equilibrium, maximum 28 days (isophorone: 14 days), followed by:
- transfer to aquarium with pollutant-free water;
- further duration 7 days
ANALYSES
- samples of 5 ml water and 5 fish each on days 1, 2, 4, 7, 10, 14 (other substances: also 21 and 28) of exposure; control water on days 0, 28;
- samples of 5 fish on days 1, 2, 4, and 7 of depuration;
- weighing of fish (result refers to wet weight);
- combustion of fish;
- trapping of 14CO2 in solution with scintillators;
- counting in scintillation spectrometer

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(142)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : .5 mg/l
BCF : = 1.1 - 1.8
Elimination : no data
Method : other: corresponding to OECD guideline 305 C
Year : 1992
GLP : no data
Test substance : no data

Result : BCF at 0.05 mg/l exposure level < 10
Test condition : Flow-through system; O2 6-8 mg/l; 15-20 fish/level; Analysis by gas chromatography
Reliability : (2) valid with restrictions
Test procedure according to guideline without detailed documentation

Flag : Critical study for SIDS endpoint

(96)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	
LC50	:	= 228
EC50	:	= 217
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other
Year	:	1990
GLP	:	no
Test substance	:	other TS: purity 97%, Aldrich Chemical Co.
Method	:	Fish (27-74 days old; mean lenght 15.5 mm; mean weight: 0.052 g) exposed in Lake Superior water; 5 TS concentrations in the range of 46.5 to 213 mg/l tested (plus control); number of fish recorded every 24 h; observations of fish behaviour and body morphology at regular intervals; TS analysis by GLC
Result	:	No confidence limits estimated Affected fish lost schooling behavior, were hypoactive and underreactive to external stimuli and were darkly colored. They had also spinal column deformities and lost equilibrium prior to death.
Test condition	:	DILUTION WATER - Alkalinity: 40.5 mg/l CaCO3 - Hardness: 50.0 mg/l CaCO3 - pH: 7.5 - Oxygen content: 7.1 mg/l TEST SYSTEM - Test type: flow through - Concentrations: 46.5, 71.5, 110, 170, 261 mg/l (nominal) 43.8, 72.0, 114, 186, 275 mg/l (measured) - Number of replicates: 2 - fish per replicate: 5-25 - Test temperature: 24.5 degrees C - Adjustment of pH: adjusted to lake water using NaOH - Photoperiod: 16 h light
Reliability	:	(1) valid without restriction Test procedure comparable to standard method and in accordance with general accepted scientific standards; detailed documentation of test procedure and test conditions
Flag	:	Critical study for SIDS endpoint

(40)

Type	:	flow through
Species	:	Cyprinodon variegatus (Fish, estuary, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 140
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other: Acute Toxicity Test based on a procedure of the Committee on Methods for Toxicity Tests with Aquatic Organisms
Year	:	1981
GLP	:	no data
Test substance	:	no data

Remark : Analytical concentrations were 149-187 % of nominal.
Results refer to mean measured concentration.
Result : 95 % confidence limits of LC50: 90-217 mg/l
Test condition : TEST ORGANISMS
- Supplier: inhouse (Bionomics Marine Research Laboratory)
- Age/size/weight/loading: < 20 days
- Pretreatment: in natural, flowing seawater at 30 +/- 1 degree C

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: not used
- Other procedures: diluter cycled for >= 24 h in advance

DILUTION WATER
- Source: natural sea water, Big Lagoon (estuary of Gulf of Mexico), filtered
- Salinity: daily (few exceptions)
- pH: daily (few exceptions)
- Oxygen content: daily (few exceptions)

TEST SYSTEM
- Concentrations: 5 levels, 47-608 mg/l
- Dosing rate: 1 l per cycle
- Renewal of test solution: 4-7 cycles per hour
- Exposure vessel type: glass aquariums 30 cm wide, 30 cm high, 91 cm long, containing approximately 41 l solution
- Number of replicates, fish per replicate: not reported
- Test temperature: not reported
- Dissolved oxygen: not reported
- pH: not reported
- Adjustment of pH: no
- Intensity of irradiation: approximately 1100 lux incident to water surface
- Photoperiod: 16 hours light, 8 hours dark

TEST PARAMETER: mortality
MONITORING OF TEST SUBSTANCE CONCENTRATION: 50 ml each at beginning and end of test, frozen until analysis, filtered, bottles rinsed with 5 ml methanol, combined phase directly analyzed by liquid chromatography
STATISTICAL ANALYSIS: conversion of concentrations to log and of percentages mortality to probit
Test substance : commercial, no details reported
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint

(144)

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 255
Limit test : no
Analytical monitoring : yes
Method : other: Acute Toxicity Test
Year : 1982
GLP : no data
Test substance : no data

Method : Stephan, C.E. (1975): Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser. EPA-660/3-75-009 Corvallis, OR: U.S. Environ. Prot. Agency

Remark : Among the two values of unrestricted validity, the one which was more unlike an early life stage test was selected.
Result : 95 % confidence interval (LC50): 229-283 mg/l
Test condition : TEST ORGANISMS
- Age/size/weight/loading: Initial age 6-8 weeks

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dispersion: Diluter
- Vehicle, solvent: none

DILUTION WATER
- Source: Western Fish Toxicology Station, Corvallis, Oregon
- Alkalinity: 28 (20-47) mg/l CaCO3
- Hardness: 35 (24-70) mg/l CaCO3
- pH: 7.4 (7.1-7.6)
- Oxygen content: 7.9 (6.2-8.4) mg/l

TEST SYSTEM
- Concentrations: 73-412 mg/l
- Renewal of test solution: 88 volume additions in 24 hr
- Exposure vessel type: 1 l glass beakers, 900 ml solution, 10.5 cm deep
- Number of replicates, fish per replicate: 2 replicates, 10 fish per chamber
- Test temperature: 24.5 +/- 1 degree C
- Dissolved oxygen: 7.9 (6.2-8.4) mg/l
- pH: 7.4 (7.1-7.6)
- Intensity of irradiation: approximately 270 lux on surface
- Photoperiod: 16 h light / 8 h dark

MONITORING OF TEST SUBSTANCE CONCENTRATION: twice weekly, HPLC

STATISTICAL METHODS: trimmed Spearman-Karber method
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(14)

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 145
Limit test : no
Analytical monitoring : yes
Method : other: Acute Toxicity Test
Year : 1982
GLP : no data
Test substance : no data

Method : Stephan, C.E. (1975): Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser. EPA-660/3-75-009 Corvallis, OR: U.S. Environ. Prot. Agency
Result : 95 % confidence interval (LC50): 132-159 mg/l
Test condition : TEST ORGANISMS
- Age/size/weight/loading: Initial age 3 weeks

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dispersion: Diluter
- Vehicle, solvent: none

DILUTION WATER
- Source: Western Fish Toxicology Station, Corvallis, Oregon
- Alkalinity: 28 (20-47) mg/l CaCO3
- Hardness: 35 (24-70) mg/l CaCO3
- pH: 7.4 (7.1-7.6)
- Oxygen content: 7.9 (6.2-8.4) mg/l

TEST SYSTEM
- Concentrations: 59-394 mg/l
- Renewal of test solution: 88 volume additions in 24 hr
- Exposure vessel type: 1 l glass beakers, 900 ml solution, 10.5 cm deep
- Number of replicates, fish per replicate: 2 replicates, 10 fish per chamber
- Test temperature: 24.5 +/- 1 degree C
- Dissolved oxygen: 7.9 (6.2-8.4) mg/l
- pH: 7.4 (7.1-7.6)
- Intensity of irradiation: approximately 270 lux on surface
- Photoperiod: 16 h light / 8 h dark

MONITORING OF TEST SUBSTANCE CONCENTRATION: twice weekly, HPLC

STATISTICAL METHODS: trimmed Spearman-Karber method

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(14)

Type	:	other: static or semi-static
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	= 340
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: according to JIS K 0102-1986-71 (Japanese Industrial Standard)
Year	:	1992
GLP	:	no data
Test substance	:	no data

Test condition : 25 +- 2 degrees C; 10 fish/level
Reliability : (2) valid with restrictions
Test procedure according to guideline without detailed documentation

(96)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 130
LC50	:	= 209
LC100	:	= 300
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: DIN 38412 part 15
Year	:	1996
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Remark	:	The test was performed in March 1983.
Result	:	130 mg/l: 0 % dead 160 mg/l: 20 % dead 200 mg/l: 40 % dead 250 mg/l: 70 % dead 300 mg/l: 100 % dead confidence interval of LC50: 200-250 mg/l
Test condition	:	TEST ORGANISMS - Strain: Leuciscus idus melanotus HECKEL - Supplier: Eggers, Hohenwestedt - Wild caught: no - Age/size/weight/loading: 6 +/- 2 cm - Feeding: daily 3 % of body weight TetraMin - Pretreatment: single treatment with Zephirol 1:50,000 for 1 hour followed by 14 days under quarantine - Feeding during test: no STOCK AND TEST SOLUTION AND THEIR PREPARATION - Concentration of vehicle / solvent: 10 g test substance/l, no vehicle or solvent DILUTION WATER - Source: dechlorinated drinking water - Aeration: continuous - Hardness: ca. 15 degree dH TEST SYSTEM - Test type: static - Concentrations: 130 / 160 / 200 / 250 / 300 mg/l - Renewal of test solution: no - Exposure vessel type: 10 l solution in 18 l aquarium - Number of replicates, fish per replicate: 1, 10 - Test temperature: 20 +/- 1 degree C - Dissolved oxygen: 4.7-7.0 mg/l - pH: 7.4-8.3 - Photoperiod: 8 / 16 hours
Test substance	:	Huels AG
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods with acceptable restrictions

(65)

Type	:	static
Species	:	Cyprinodon variegatus (Fish, estuary, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 170
LC50	:	170 - 300
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians, US EPA
Year	:	1981
GLP	:	no data
Test substance	:	other TS
Test condition	:	TEST ORGANISMS - Supplier: Bionomics Marine Res. Lab., Pensacola, FL, or U.S. EPA Environ. Res. Lab., Gulf Breeze, FL - Age/size/weight/loading: 14-28 days post hatch; length 8-15 mm - Feeding: 24-h Artemia salina nauplii, daily before test - Feeding during test: no

DILUTION WATER
- Source: 5 um filtered ambient salinity natural sea water
- Aeration: no
- Salinity: 10-31 o/oo
TEST SYSTEM
- Exposure vessel type: glass jar
- Number of replicates, fish per replicate: 10 fish / jar
- Test temperature: 25-31 degree C

Test substance : 54 analytical grade substances with purities >= 80 % tested

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(51)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 220
Limit test : no
Analytical monitoring : no
Method : other: Methods for Acute Toxicity Test with Fish, Macroinvertebrates, and Amphibians, US EPA
Year : 1975
GLP : no data
Test substance : other TS

Result : LC50 (24 h) = 240 mg/l
95 % confidence interval of LC50 (96 h): 180-250 mg/l

Test condition : TEST ORGANISMS
- Supplier: commercial, USA, identity not reported
- Age/size/weight/loading: "young of the year", 0.32-1.2 g
- Feeding: ad libitum daily with dry, pelleted food;
once weekly with raw ground beef liver
discontinued 48 h prior to testing
- Feeding during test: no
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: 1,6-hexanediol; acetone;
dimethyl formamide; ethanol (order of preference)
DILUTION WATER
- Source: based on deionized water,
prepared according to U.S. EPA procedures (1975)
- Alkalinity: 28-34 mg CaCO3/l
- Hardness: 32-48 mg CaCO3/l
- pH: 6.7-7.8
- Oxygen content: 7.0-8.8 mg/l
- Conductance: 93-190 umhos/cm
TEST SYSTEM
- Renewal of test solution: no
- Exposure vessel type: 19.6 l widemouthed glass jars containing 15 l of test solution, capped
- Number of replicates, fish per replicate: 10 fish
- Test temperature: 22 +/- 1 degree C
CALCULATION METHOD:
Moving average angle method (Harris 1959) if applicable,
otherwise log probit method
results based on nominal concentrations

Test substance : >= 80 % pur; purest grade commercially available (summary statements for all test substances)

Reliability : (3) invalid
Significant methodological deficiencyi: Undissolved

chemical was observed. In view of the high water solubility of isophorone, this was probably an impurity.

(11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 15
LC50	:	= 120
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians, US EPA (1975)
Year	:	1980
GLP	:	no data
Test substance	:	other TS
Result	:	95 % confidence interval: 72-170 mg/l 24 h LC50 = 430 mg/l; 95 % confidence interval: 360-500 mg/l
Test condition	:	TEST ORGANISMS - Source/supplier: EG&G Bionomics inhouse - Age: < 24 hours - Control group: dilution water STOCK AND TEST SOLUTION AND THEIR PREPARATION - Vehicle, solvent: not reported, probably none - Other procedures: Preparation depended on solubility. Procedures are reported but not assigned to substances. DILUTION WATER - Hardness: 72 mg/l as CaCO3 - pH: 7.0 +/- 0.2 - Oxygen content: > 60 % at study initiation TEST SYSTEM - Concentrations: 5 to 8, values not reported - Renewal of test solution: no - Exposure vessel type: 250 ml beaker - Number of replicates, individuals per replicate: 3, 5 - Test temperature: 22 +/- 1 degree C - Dissolved oxygen: 6.5-9.1 mg/l - pH: 6.7 - 8.1 - Adjustment of pH: no
Test substance	:	78 commercial substances with purities >= 80 % tested
Reliability	:	(2) valid with restrictions Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

27.06.2003 (79)

Type	:	static
Species	:	Mysidopsis bahia (Crustacea)
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	= 12.9
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other
Year	:	

GLP	:	no data
Test substance	:	no data
Remark	:	Original reference: "U.S. EPA (1978), In-depth studies on health and environmental impacts of selected water pollutants. Contract No. 68-01-4646." No final report existing.
Reliability	:	(4) not assignable Test protocol not available; documentation insufficient for assessment; cited in peer-reviewed substance dossier (139) (146)
Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	= 90
EC50	:	= 254
EC100	:	= 500
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: DIN 38412 part 11
Year	:	1996
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The test was performed in March 1988.
Result	:	- Concentration / response table: 60 / 90 mg/l: 0 % immobile 120 / 180 mg/l: 15 % immobile 250 mg/l: 50 % immobile 350 mg/l: 65 % immobile 500 / 700 / 1000 mg/l: 100 % immobile 95 % confidence interval of LC50: 221-293 mg/l (probit analysis, Cavalli-Sforza 1972) RESULTS: TEST WITH REFERENCE SUBSTANCE - Concentrations: 0.9 / 1.9 mg/l - Results: 5 / 95 % immobilisation
Test condition	:	TEST ORGANISMS - Strain: Daphnia magna, Huels - Source/supplier: Huels AG (inhouse) - Breeding method: in 1 l jars with dechlorinated drinking water, water renewal each 2-3 days, isolation of juveniles for further breeding each ca. 4 weeks - Age: < 24 hours - Feeding: Chlorella vulgaris, as much as consumed - Pretreatment: Filtration of adults 24 h prior to testing - Feeding during test: no - Control group: 2 reference substance controls, one blank STOCK AND TEST SOLUTION AND THEIR PREPARATION - Concentration: 2 g/l, no solvent or vehicle REFERENCE SUBSTANCE: potassium dichromate, CAS RN 7778-50-9 DILUTION WATER - Source: Synthetic: CaCl2 x 2 H2O: 294 mg/l MgSO4 x 7 H2O: 123 mg/l NaHCO3: 63 mg/l KCl: 5.5 mg/l - Ca/Mg ratio: 4:1 - Na/K ratio: 10:1 TEST SYSTEM

- Concentrations:
  60; 90; 120; 180; 250; 350; 500; 700; 1000 mg/l
- Exposure vessel type: 25 ml graduated cylinder
- Number of replicates, individuals per replicate:
  4 replicates with 5 individuals each
- Test temperature: 20 +/- 1 degree C
- Intensity of irradiation: dark
- Photoperiod: -

DURATION OF THE TEST: 24 hours
TEST PARAMETER: immobilisation

Test substance : Huels AG, Sample 530/880202

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions

(66)

Type : static
Species : Artemia salina (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = 430
Limit Test : no
Analytical monitoring : no data
Method : other: Acute Toxicity Test
Year : 1974
GLP : no data
Test substance : no data

Test condition : TEST ORGANISMS
- Source/supplier: dried eggs from Carolina Biological Supply Co., Burlington, N.C.
- Breeding method: aeration in synthetic seawater until hatching was completed; settling out of unhatched eggs, concentration of shrimps in beam of light and transport to separate container
- Control group: synthetic seawater
- Age: 48 hours

DILUTION WATER
- Synthetic seawater:
  557.37 g NaCl, 27.20 g CaSO4, 63.36 g MgSO4 x 7 H2O, 168.30 g MgCl2, 15.84 g KCl, 3.14 g MgBr2 x 6 H2O, all dissolved in 20 l of distilled water in this order

TEST SYSTEM
- Concentrations: 100, 180, 320, 560, 1000 mg/l (selection based on screening test)
- Renewal of test solution: no
- Exposure vessel type: 150 ml wide-mouth bottles
- Number of replicates, individuals per replicate: 1; 30-50
- Test temperature: 24.5 degree C

DURATION OF THE TEST: 24 hours
TEST PARAMETER: no movement of the phyllopodia

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions

(106)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)

Endpoint	:	biomass
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC10	:	= 64
EC50	:	= 475
EC90	:	> 1000
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: Growth Inhibition Test according to a proposal of the Umweltbundesamt, Germany
Year	:	1988
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Turbidity after 0 / 24 / 48 / 72 h: Control: 0.5 / 1.8 / 4.4 / 8.9 125 mg/l: 0.5 / 1.9 / 3.5 / 6.4 200 mg/l: 0.5 / 1.9 / 3.5 / 5.8 350 mg/l: 0.5 / 2.1 / 2.5 / 4.1 600 mg/l: 0.5 / 2.1 / 1.9 / 1.9 1000 mg/l: 0.5 / 1.8 / 1.6 / 2.2 STATISTICAL RESULTS: comparison of areas under growth curves, probit transformation, probit analysis (Cavalli-Sforza 1972)
Test condition	:	TEST ORGANISMS - Strain: CHODAT (86.81 SAG) - Source/supplier: Origin: Institut fuer Wasser-, Boden- und Lufthygiene, Berlin, further bred inhouse - Laboratory culture: From a stock culture, a preculture is seeded three days before begin of test. Test cultures are seeded from the latter. - Method of cultivation: Erlenmeyer flasks on tables exposed to light - Controls: yes - Initial cell concentration: ca. 20,000 cells/ml STOCK AND TEST SOLUTION AND THEIR PREPARATION - Concentration of vehicle/ solvent: 10 g test substance/l, no vehicle or solvent DILUTION WATER - Aeration: yes, sterile TEST SYSTEM - Concentrations: 0 / 125 / 200 / 350 / 600 / 1000 mg/l - Renewal of test solution: no - Number of replicates: 3
Test substance	:	Huels AG, Sample 530/880202
Reliability	:	(2) valid with restrictions Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(67)

Species	:	other algae: Champia parvula (marine red macroalgae)
Endpoint	:	other: growth and reproduction
Exposure period	:	14 day(s)
Unit	:	mg/l
NOEC	:	= 29.9
LOEC	:	= 49.8
MATC	:	= 38.6
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: see Test Conditions

Year	:	1983
GLP	:	no data
Test substance	:	no data
Remark	:	LOEC: lowest concentration that was statistically different from the control in tetrasporophytes
Result	:	LOECs for various endpoints: - vegetative growth (dry weight): 83.07 mg/l - sexual reproduction (cystocarps): 83.07 mg/l - asexual spore production (tetrasporangia): 49.84 mg/l NOEC = 60% of LOEC
Test condition	:	TEST ORGANISMS - Strain: Champia parvula (C. Agardh) Harvey - Laboratory culture: inhouse - Method of cultivation: 1000 ml Erlenmeyer flasks containing 800 ml of culture medium; culture medium made from natural seawater to which additional nutrients (as below, but 4/4 portions of vitamin solution) were added after filtration and autoclave treatment - Pretreatment: stock cultures started weekly, halved weekly with change of media, ready after 3 weeks; 5 female branch tips (2-3 mm) plus one male (1 cm) branch per test vessel - Controls: yes STOCK AND TEST SOLUTION AND THEIR PREPARATION - Vehicle, solvent: none GROWTH/TEST MEDIUM CHEMISTRY - Salinity: 30 o/oo - EDTA: no - added per liter of filtered seawater: 37.4 mg NaNO3, 2.5 mg NaH2PO4 x H2O, 10.4 ug Fe (as FeCl2) 1/4 portion of: (0.24 ug vitamin B12, 0.24 ug biotin, 50 ug thiamine x HCl) TEST SYSTEM - Concentrations: dilution factor 0.6; maximum determined in screening test - Renewal of test solution: days 7 and 11 - Exposure vessel type: 500 ml Erlenmeyer flasks filled 80 %, screw-capped - Number of replicates: 2 - Test temperature: 22-24 degree C - Intensity of irradiation: 75 uE/(m2 s) - Photoperiod: 16 h light / 8 h dark - Aeration: substituted by 150 mg/l sodium bicarbonate - Duration: 11 (for tetrasporangia) or 14 (for females) days TEST PARAMETER: - vegetative growth (dry weight after 48 h at 80 degree C); - sexual reproduction (development of cystocarps); - asexual spore production (formation of tetrasporangia) - MATC = sqrt (LOEL x NOEL) for most sensitive endpoint
Reliability	:	(2) valid with restrictions No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(134)

Species	:	Skeletonema costatum (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l

EC50	:	= 105
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other
Year	:	1978
GLP	:	no data
Test substance	:	no data
Remark	:	Original reference: "U.S. EPA (1978), In-depth studies on health and environmental impacts of selected water pollutants. Contract No. 68-01-4646." No final report existing.
Result	:	Endpoint Chlorophyll: EC50 = 110 mg/l
Reliability	:	(4) not assignable Test protocol not available; documentation insufficient for assessment; cited in peer-reviewed substance dossier (139) (146)
Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	= 122
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: Toxicity to Algae
Year	:	1978
GLP	:	no data
Test substance	:	no data
Remark	:	Original reference: "U.S. EPA (1978), In-depth studies on health and environmental impacts of selected water pollutants. Contract No. 68-01-4646." No final report existing.
Result	:	Endpoint Chlorophyll: EC50 = 126 mg/l
Reliability	:	(4) not assignable Test protocol not available; documentation insufficient for assessment; cited in peer-reviewed substance dossier (139) (146)
Species	:	other algae: Champia parvula (marine red macroalgae)
Endpoint	:	other: sexual reproduction
Exposure period	:	14 day(s)
Unit	:	mg/l
EC100	:	= 107.3
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: Sexual Reproduction Test
Year	:	1983
GLP	:	no data
Test substance	:	no data
Test condition	:	TEST ORGANISMS - Strain: Champia parvula (C. Agardh) Harvey - Laboratory culture: inhouse - Method of cultivation: 1000 ml Erlenmeyer flasks containing 800 ml of culture medium; culture medium made from natural seawater to which additional nutrients (as below, but 4/4 portions of vitamin solution) were added after filtration and autoclave treatment - Pretreatment: stock cultures started weekly, halved weekly with change of media, ready after 3 weeks; 5 female branch tips (2-3 mm) plus one male branch per test vessel

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: none
GROWTH/TEST MEDIUM CHEMISTRY
- Salinity: 28-30 o/oo
- EDTA: no
- added per liter of filtered seawater:
37.4 mg NaNO3,
2.5 mg NaH2PO4 x H2O,
10.4 ug Fe (as FeCl2)
1/4 portion of:
(0.24 ug vitamin B12, 0.24 ug biotin, 50 ug thiamine x HCl)
TEST SYSTEM
- Concentrations: dilution factor 0.6; maximum determined in screening test
- Renewal of test solution: days 7 and 11
- Exposure vessel type: 500 ml Erlenmeyer flask filled 80 %, screw-capped
- Number of replicates: 2
- Test temperature: 22-24 degree C
- Intensity of irradiation: 75 uE/(m2 s)
- Photoperiod: 16 h light / 8 h dark
- Aeration: replaced by 150 mg/l sodium bicarbonate
TEST PARAMETER: sexual reproduction (development of cystocarps)

Test substance : commercial, used directly

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(133)

Species : other algae: Champia parvula (marine red macroalgae)
Endpoint : other: sexual reproduction
Exposure period : 2 day(s)
Unit : mg/l
EC50 : = 38.3
EC100 : > 138.5
EC95 : = 107.3
Limit test : no
Analytical monitoring : no
Method : other: Sexual Reproduction Test
Year : 1986
GLP : no data
Test substance : no data

Test condition : TEST ORGANISMS
- Strain: Champia parvula (C. Agardh) Harvey
- Laboratory culture: inhouse
- Method of cultivation: 1000 ml Erlenmeyer flasks containing 800 ml of culture medium; culture medium made from natural seawater to which additional nutrients (as below, but 4/4 portions of vitamin solution) were added after filtration and autoclave treatment
- Pretreatment: stock cultures started weekly, halved weekly with change of media, ready after 3 weeks; 5 female branch tips (7-10 mm) plus one male branch per test vessel
- Controls: yes
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: none
GROWTH/TEST MEDIUM CHEMISTRY
- Salinity: 28-30 o/oo
- EDTA: no
- added per liter of filtered seawater:

37.4 mg NaNO3,
2.5 mg NaH2PO4 x H2O,
10.4 ug Fe (as FeCl2)
1/4 portion of:
(0.24 ug vitamin B12, 0.24 ug biotin, 50 ug thiamine x HCl)
TEST SYSTEM
- Concentrations: dilution factor 0.6; maximum determined in screening test
- Exposure vessel type: 100 ml polystyrene cups filled 80 %, capped
- Number of replicates: 2
- Test temperature: 22-24 degree C
- Intensity of irradiation: 75 uE/(m2 s)
- Photoperiod: 16 h light / 8 h dark
- Aeration: only during recovery period
- Duration: 2 days exposure followed by 7-9 days for recovery
TEST PARAMETER: sexual reproduction (development of cystocarps)

Test substance : commercial, used directly

Reliability : (3) invalid
Significant methodological deficiencies: Test substances may interact with the test vessel (polystyrene). Problems met with test substance benzene were not clarified satisfactorily.

(133)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	activated sludge of a predominantly domestic sewage
Exposure period	:	3 hour(s)
Unit	:	mg/l
EC50	:	= 100
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 209 "Activated Sludge, Respiration Inhibition Test", Draft 1.8.83
Year	:	1986
GLP	:	no data
Test substance	:	no data

Test condition :
TEST ORGANISMS
- Origin: Gifu-Hokubu Sewage Treatment Plant
- Pretreatment: washing with dist. water >= 3 times, suspended in dist. water at 4 g/l
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dispersion:
- Vehicle, solvent: dimethyl sulfoxide/surfactant HCO-40 (4:1)
- Concentration of vehicle/ solvent: 2.0 g/l
- Other procedures: Synthetic sewage feed: 16 g/l peptone, 11 g/l meat extract, 3 g/l urea, 0.7 g/l NaCl, 0.4 g/l CaCl2 x 2 H2O, 0.2 g/l MgSO4 x 7 H2O, 2.8 g/l K2HPO4 in dist. water
REFERENCE SUBSTANCE: 3,5-dichlorophenol
TEST SYSTEM
- Concentrations: at least five, factor 3.2
- Exposure vessel type: 1 l beaker filled as follows: 16 ml sewage feed, stock solution (volume determined by desired

concentration), made up to 300 ml with dist. water, 200 ml activated sludge added
- Test temperature: 20 degree C
- Aeration: 1 l/min with Pasteur pipette
- Control: one each at beginning and end of test series (mean used as reference in determination of inhibition)
TEST PARAMETER: oxygen consumption after this procedure, measured over 10 min in 300 ml Erlenmeyer flask; graphical evaluation on log-normal paper

Reliability : (2) valid with restrictions
Comparable to guideline study

Flag : Critical study for SIDS endpoint

(148)

Type : aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period : 18 hour(s)
Unit : mg/l
EC10 : = 340 - 530
Analytical monitoring : no
Method : other: Bringmann and Kuehn, Z. Wasser Abwasser Forsch. 10, 87-98 (1977)
Year : 1988
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The test was performed in Feb 1988.
Result : mean EC10 = 435 mg/l
Test condition : - Number of replicates:
5 growth controls
4 controls
3 replicates of 4 concentrations
all performed twice
- Test vessel: 250 ml Erlenmeyer flasks, sterile, capped with cellulose
- Test concentration: 200 / 400 / 800 / 1,600 mg/l
- Duration of test: 18 +/- 1 hous
- Temperature: 25 +/- 2 degree C
- Quantification: photometric determination of turbidity at 436 nm, graphical evaluation

Test substance : Huels AG
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(68)

Type : aquatic
Species : Tetrahymena pyriformis (Protozoa)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = 420
Analytical monitoring : no data
Method : other: Cell Multiplication Inhibition Test
Year : 1985
GLP : no data
Test substance : other TS: analytical grade

Test condition : TEST ORGANISMS
- Pretreatment: basic culture in sterile medium of 2 % proteose peptone at 20 degree C which is renewed at 2-4 week intervals, pre-culture for test: 30 degree C for 24 hours

- Feeding during test: 2 % proteose peptone
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: not required
REFERENCE SUBSTANCE:
TEST SYSTEM
- Test type: static
- Renewal of test solution: no
- Exposure vessel type: not reported; 10 ml test solution
- Test temperature: 30 degree C
- Control: blank
TEST PARAMETER: number of cells (microscope and / or Coulter counter)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(149)

Type :
Species : Saccharomyces cerevisiae (Fungi)
Exposure period :
Unit : g/l
EC0 : >= 114
Analytical monitoring : no data
Method : other: Oxygen Electrode Respiration Test
Year : 1990
GLP : no data
Test substance : no data

Result : Neither inhibition of electron transfer nor uncoupling of oxidative phosphorylation took place at any tested concentration.

Test condition : TEST ORGANISMS
- Strain: S. cerevisiae C 276 a/alpha
- Source/supplier: Pringle, Univ. of Michigan
- Method of cultivation: grown on YM-1 medium with ethanol as carbon source at 37.5 degree C
- Pretreatment: centrifugation at 1000 g for 5 min, resuspension in medium (0.1 M potassium phosphate in 2 % ethanol, pH 5.8, washing twice by centrifugation as above, stored at 0-4 degree C, sonication before use)
- Controls: yes
- Initial cell concentration: 4 000 000 cells/l
STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: direct injection of test substance after the initial respiration rate of the yeast suspension had stabilized
REFERENCE SUBSTANCE: sodium sulfite
GROWTH/TEST MEDIUM CHEMISTRY
0.1 M potassium phosphate in 2 % ethanol
- pH: 5.8
- Dissolved oxygen: 7.02 mg/l
TEST SYSTEM
- Concentrations: 1.97-824 umol/l (0.27-114 g/l)
- Renewal of test solution: no
- Exposure vessel type: 2.5 l solution; vessel unspecified
- Test temperature: 30 +/- 0.2 degree C
- Exposure period: minutes
- Pressure: 750 +/- 5 Torr
TEST PARAMETER:
dissolved oxygen / respiration inhibition

Reliability : (2) valid with restrictions

No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

(49)

4.5.1 CHRONIC TOXICITY TO FISH

Species	:	Pimephales promelas (Fish, fresh water)
Endpoint	:	weight of young fish
Exposure period	:	35 day(s)
Unit	:	mg/l
LLC	:	= 112
NOEC	:	= 11
LOEC	:	= 19
Analytical monitoring	:	yes
Method	:	other: Early Life Stage Test
Year	:	1982
GLP	:	no data
Test substance	:	no data

Method : Stephan, C.E. (1979): Guidelines for conducting flow-through early life stage toxicity tests with fathead minnows for use in the U.S. EPA, OTS-ORD round robin test. Duluth, MN: U.S. Environ. Prot. Agency

Result : Survival: LOEL = 112 mg/l; NOEL = 56 mg/l
Fork length: LOEL = 30 mg/l; NOEL = 19 mg/l
Weight: LOEL = 19 mg/l; NOEL = 11 mg/l
Geometric mean NOEL = 14 mg/l

Test condition : TEST ORGANISMS
- Age/size/weight/loading: embryos <= 48 hours old

- Pretreatment: eggs from spawning substrates; dead and unfertilized eggs were discarded

- Feeding during test: live, newly-hatched brine shrimp at least twice daily on weekdays and once daily on weekends

- Controls: freshwater

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dispersion: Diluter
- Vehicle, solvent: none

DILUTION WATER
- Source: Western Fish Toxicology Station, Corvallis, Oregon
- Alkalinity: 28 (20-47) mg/l CaCO3
- Hardness: 35 (24-70) mg/l CaCO3
- pH: 7.4 (7.1-7.6)
- Oxygen content: 7.9 (6.2-8.4) mg/l

TEST SYSTEM
- Concentrations: 112 +/- 15; 56 +/-13; 30 +/- 11; 19 +/- 9; 11 +/- 6 mg/l
- Renewal of test solution: 88 volume additions in 24 hr
- Exposure vessel type: 1 l glass beakers, 900 ml solution, 10.5 cm deep, 5.7 cm square embryo scren trays
- Number of replicates, fish per replicate: 4 replicates, about 8 embryos per chamber
- Test temperature: 24.5 +/- 1 degree C

- Dissolved oxygen: 7.9 (6.2-8.4) mg/l
- pH: 7.4 (7.1-7.6)
- Intensity of irradiation: approximately 270 lux on surface
- Photoperiod: 16 h light / 8 h dark

DURATION OF THE TEST: 35 days

MONITORING OF TEST SUBSTANCE CONCENTRATION:
twice weekly, HPLC

STATISTICAL METHODS: two-way analysis of variance (ANOVA) after arcsin transformation (for percentages) or conversion to logs (for weights and lengths); Williams multiple comparison test for differences among treatments (P = 0.05)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(14)

Species	:	Pimephales promelas (Fish, fresh water)
Endpoint	:	weight of young fish
Exposure period	:	32 day(s)
Unit	:	mg/l
NOEC	:	= 4.2 - 15.6
LOEC	:	= 8.8 - 22.7
Analytical monitoring	:	yes
Method	:	other: Flow-Through Early Life Stage Test, US EPA proposal
Year	:	1983
GLP	:	no data
Test substance	:	no data

Method : Interlaboratory comparison test with a draft test procedure.
2 tests conducted according to the same test procedure, only difference in the feeding regime

Result : Test 1: NOEC (weight) = 15.6 mg/l
Test 2: NOEC (weight) = 4.2 mg/l
Feeding was identified to be crucial for the test. The total growth of fish was higher when the test was started early during the week (monday / tuesday) because then fish were fed three times daily during the first days.

Test condition : TEST ORGANISMS
- Post-hatch transfer time: < 24 hours
- Age: < 24 hours
- Feeding: Artimia salina nauplii (sufficient, some not eaten), three times daily, on weekends only two times daily
- Feeding during test: After 1 week additionally 5 g trout starter/day
- Controls: yes, dilution water

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: none; pure substance added to water

DILUTION WATER
- Source: sand filtered Lake Superior water
- Alkalinity: 40-42 mg/l as CaCO3
- Hardness: 45-47 mg/l as CaCO3
- pH: 7.8

TEST SYSTEM
- Test type: flow-through
- Concentrations: 5 per test (2.14 / 4.18 / 8.29 / 15.61 / 22.66 mg/l and 2.18 / 4.15 / 8.78 / 14.51 / 27.63 mg/l)
- Renewal of test solution: 25 fold in 24 hours

- Exposure vessel type: days 1-4: glass embryo cup; then release to glass vessel 46 cm x 16 cm x 18 cm, filled with 10 cm deep water = ca. 8.25 l;
- Number of replicates, individuals per replicate: 2, 15-35
- Test temperature: twice weekly, 24.2-25.6 (mean 25.1) degree C
- Dissolved oxygen: once/week, always > 90 %
- pH/hardness/alkalinity: twice during test, 7.5-7.8/47-48 mg/l as CaCO3/38-40 mg/l as CaCO3

MONITORING OF TEST SUBSTANCE CONCENTRATION: 1st day, twice weekly, last day: extraction with hexane, GC analysis, quantification with external standard

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(82)

Species : Cyprinodon variegatus (Fish, estuary, marine)
Endpoint : length of young fish
Exposure period : 28 day(s)
Unit : mg/l
NOEC : = 80
LOEC : = 156
Analytical monitoring : yes
Method : other: Early Life Stage Test based on a Procedure of the Committee on Methods for Toxicity Tests with Aquatic Organisms
Year : 1981
GLP : no data
Test substance : no data

Remark : Results refer to mean measured concentration

Result :

	mortality	hatching	length
control	4 %	83 %	11 mm
18 mg/l	0 %	86 %	11 mm
40 mg/l	18 %	83 %	12 mm
80 mg/l	8 %	79 %	11 mm
156 mg/l	25 %	75 %	4 mm
287 mg/l	100 %	20 %	-

NOEC / LOEC (hatching) = 156 mg/l / 287 mg/l
- Increased length at 40 mg/l may be due to increased availability of food caused by high mortality.
- Adverse effects:
  one two headed embryo at 18 mg/l, died after 1 day
  one eyeless juvenile at 40 mg/l, survived

Test condition : TEST ORGANISMS
- Supplier: inhouse (Bionomics Marine Research Laboratory)
- Wild caught: mothers from Big Lagoon (estuary of Gulf of Mexico)
- Post-hatch transfer time: eggs within 4 h after visual confirmation of fertilization; embryos daily
- Pretreatment: egg production of mother females enhanced by injections of human chorionic gonadotropin on 2 consecutive days; fertilization by addition of sperm suspension made from macerated testes excised from adult male fish
- Feeding during test: live Artemia salina nauplii daily
- Controls: dilution water

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: not used
- Other procedures: diluter cycled for >= 24 h in advance

STABILITY OF THE TEST CHEMICAL SOLUTIONS: analytical

concentrations averaged only 64 % of nominal concentrations, possibly due to losses by volatilization
DILUTION WATER
- Source: natural sea water, Big Lagoon, filtered
- Salinity: daily (few exceptions), 22-28 per mil
- pH: daily (few exceptions)
- Oxygen content: daily (few exceptions)
TEST SYSTEM
- Test type: flow-through
- Concentrations: (mg/l)
nominal: 0 / 30 / 59 / 119 / 238 / 476
analytical: not det. / 18 / 40 / 80 / 156 / 287
- Dosing rate: 1 l per cycle
- Renewal of test solution: 4-7 cycles per hour
- Exposure vessel type: glass aquariums 30 cm wide, 30 cm high, 91 cm long, containing approximately 41 l solution; embryos in 100 ml glass jars with 425 um-square mesh nylon screens as bottoms, suspended in aquariums; juveniles in glass chambers 14 cm x 20.5 cm x 26 cm with 425 um-square mesh nylon screen over one end
- Number of replicates, fish per replicate: 2 replicates with 50 eggs each; 2 replicates with maximum 40 juveniles each
- Test temperature: 29 +/- 1 degree C
- Dissolved oxygen: 4.0-6.8 mg/l
- pH: 7.8-8.3
- Adjustment of pH: no
- Intensity of irradiation: approximately 1100 lux incident to water surface
- Photoperiod: 16 hours light, 8 hours dark
DURATION OF THE TEST: 28 days after hatch
ENDPOINTS ASSESSED: hatching success, survival, growth
MONITORING OF TEST SUBSTANCE CONCENTRATION: weekly, 50 ml each, frozen until analysis, filtered, bottles rinsed with 5 ml methanol, combined phase directly analyzed by liquid chromatography
STATISTICAL ANALYSIS: statistical significance determined by analysis of variance (ANOVA), after arcsin percentage transformations of binomial percentages to angles of equal information in degrees (hatching success, mortality); ANOVA (length); 95 % confidence level ($p < 0.05$) applied to differences between test and control solutions.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

27.06.2003 (144)

Species : Pimephales promelas (Fish, fresh water)
Endpoint : weight of young fish
Exposure period : 28 day(s)
Unit : mg/l
NOEC : 1.35 - 45.4
Analytical monitoring : yes
Method : other: Flow-Through Early Life Stage Test, US EPA proposal
Year : 1983
GLP : no data
Test substance : no data

Remark : Data are from an interlaboratory comparison test with a draft test procedure.
Growth of control fish varied vrom 0.0018 gr to 0.969 gr

indicating severe deficiencies in feeding regime.

Test condition : TEST ORGANISMS
- Age/size/weight/loading: < 24 hours
- Feeding during test: Mostly Artemia salina nauplii, twice daily except on weekends
- Controls: yes

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: used by 1 laboratory, ID not reported

DILUTION WATER
- Source: well water (4 out of 6) / other
- Aeration: not quantified
- Hardness: different between laboratories
- pH: 7.1-8.5
- Oxygen content: > 70 %

TEST SYSTEM
- Test type: flow-through
- Concentrations: 5
- Renewal of test solution: between 8 and 88 times/24 hours
- Exposure vessel type: various
- Number of replicates, individuals per replicate: 6 laboratories, each 2 replicates with about 60 individuals
- Test temperature: 25 +/- 1 degree C
- pH: 7.1-8.5

DURATION OF THE TEST: 28 days

MONITORING OF TEST SUBSTANCE CONCENTRATION: GC or UV

Reliability : (3) invalid
Significant deficiencies in feeding regime

(81)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

Species	:	other: Ampelisca abdita
Endpoint	:	Mortality
Exposure period	:	10 other: days
Unit	:	
Method	:	
Year	:	1996
GLP	:	
Test substance	:	

Method : Determination of the toxicity of sediments collected from a polluted river system

Result : Correlation between measured isophorone concentrations and mortality of test organism unclear

Test condition : Sediments collected from 38 stations
Sediment analysis: beside isophorone, 38 contaminants were identified
Test system: seawater overflows bedded sediments

Reliability : (3) invalid
Unsuitable test system

(107)

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

Type	:	other: fungi
Deg. product	:	
Result	:	Microbial transformation yielded the following metabolites: 3,5,5-trimethyl-2-cyclohexene-1,4-dione, 3,5,5-trimethylcyclohexane-1,4-dione, 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one, and 3-hydroxymethyl-5,5-dimethyl-2-cyclohexen-1-one.
Test condition	:	TEST ORGANISMS - Strain: aspergillus niger JTS 191 TEST MEDIUM 3 % sucrose, 0.2 % NaNO3, 0.1 % K2HPO4, 0.05 % KCl, 0.05 % MgSO4x7H2O, 0.1 % yeast extract in dist. water TEST SYSTEM - Concentration of isophorone: 0.1 % (wt/vol) = 1 g/l - Concentration of fungus: 4.0E+07 spores/l - Test temperature: 28 degree C - Other: Continuous shaking DURATION OF THE TEST: 48 h pretreatment, 96 h after treatment SAMPLING: daily The resulting broth was extracted with ethyl acetate, washed with 5 % NaHCO3 and concentrated. Conversion products were isolated by silicic acid column chromatography and characterised by optical activity, mass, infrared, proton NMR, and UV spectroscopy
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

(94)

4.9 ADDITIONAL REMARKS

Memo	:	Effect on crops
Remark	:	Several potential pesticide solvents were tested. A high dose was chosen to obtain visible effects.
Result	:	corn: class B; max. damage after 4 h (12.5 %) wheat: class B; max. damage after 8-56 h (10 %) cotton: class B; max. damage after 4 / 24 h (35 %) soybean: class D; max. damage after 4 h (50 %) No plant died.

Maximum damage in the overall study was observed after 32 h.
Recovery and renewed growth was observed after 56 h.
No solvent was significantly more phytotoxic than the reference solvent.
Low phytotoxicity was observed with non-polar solvents.
High phytotoxicity was observed with aromatic solvents.
The effects of surface tension (limiting contact to leaf surface) and solution potential for wax layer of leaves are discussed. For clarification further studies would be needed.

Test condition : TEST ORGANISMS
- Strain:
  cotton (Stoneville 825)
  soybean (Hutton)
  corn (H-TAM50)
  wheat (Sturdy)
- Age: 12-14 days after emergence
- Watering: dist. water with or without 0.33 ml/l "Peters professional soluble plant food, grade 20-20-20)
- Controls: yes (untreated)

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Vehicle, solvent: undiluted, i.e. no vehicle / solvent

REFERENCE SUBSTANCE: xylene range aromatic solvent

TEST SYSTEM
- Test type: one-time spray application "over the top"
- Dosing rate: 3.27 isophorone ml/m2, undiluted
- Exposure vessel type: greenhouse after application
- Number of replicates: 2

DURATION OF THE TEST:
- application at 9.27 a.m.,
  observation after 4, 8, 24, 32, 48, 56 hours

ENDPOINTS ASSESSED:
  percentage of leaf surface affected
  classification from A (minimum) to D (maximum effects)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(78)

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

Remark : There are several studies and investigations describing specific topics of the toxicokinetic and metabolism of isophorone. These studies are mentioned in chapter 5.11 (adsorption, distribution, excretion, metabolism).

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : 2100 - 2700 mg/kg bw
Species : rat
Strain : Wistar
Sex : male/female
Number of animals : 10
Vehicle : other: olive oil
Doses :
Method : other
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Result : MORTALITY:
- Time of death:
- Number of deaths at each dose:
males: LD50 = 2700 +/- 200 mg/kg
1000 mg/kg: 0/10
1500 mg/kg: 1 (within 5 hours) + 1 (day 2) = 2/10
2000 mg/kg: 7 (within 5 hours) + 1 (within 24 hours) = 8/20
3000 mg/kg: 8 (within 5 hours) + 1 (within 18 hours) = 9/20
4000 mg/kg:14 (within 5 hours) + 1 (within 24 hours) =15/20
5000 mg/kg: 9 (within 5 hours) = 9/10
females: LD50 = 2100 +/- 100 mg/kg
1000 mg/kg: 0/10
1500 mg/kg: 1 (within 5 hours) = 1/10
2000 mg/kg: 1+1+1 within 5, 18, and 24 hours) = 3/10
2500 mg/kg: 3+3+2 within 5, 18, and 24 hours) = 8/10
3000 mg/kg: 8 (within 5 hours) + 2 (within 24 hours) =10/10
CLINICAL SIGNS: weariness, leading to coma, followed by death within 24 hours or by complete recovery. Doses at which clinical signs occurred are not defined.
NECROPSY FINDINGS: no gross lesions in organs, no findings in organs of surviving animals, lesions in livers of animals that had died
POTENTIAL TARGET ORGANS: liver

Test condition : TEST ORGANISMS:
- Weight at study initiation: mean 200 g
- Number of animals per dose group:
10 in all groups except for 20 in male groups dosed 2000, 3000, and 4000 mg/kg
ADMINISTRATION: oral gavage
- Doses:

males 1000 / 1500 / 2000 / 3000 / 4000 / 5000 mg/kg
females 1000 / 1500 / 2000 / 2500 / 3000 mg/kg
- Volume administered or concentration: 1 ml / 200 g b.w.
- Post dose observation period: 14 days

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(27)

Type : LD50
Value : = 2200 mg/kg bw
Species : mouse
Strain :
Sex : male
Number of animals : 10
Vehicle : other: Olive oil
Doses :
Method : other: no data
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Result : MORTALITY:
- Time of death:
- Number of deaths at each dose:
1000 mg/kg: 0/10
1500 mg/kg: 1 (within 5 hours) = 1/10
2000 mg/kg: 5 (within 5 hours) = 5/10
2500 mg/kg: 6 (within 5 hours) = 6/10
3000 mg/kg: 7 (within 5 hours) = 7/10
4000 mg/kg:10 (within 5 hours) = 10/10
CLINICAL SIGNS: weariness, leading to coma, followed by death within 24 hours or by complete recovery
NECROPSY FINDINGS: no gross lesions in organs, no findings in organs of surviving animals, lesions in livers of animals that had died
POTENTIAL TARGET ORGANS: liver

Test condition : TEST ORGANISMS:
- Weight at study initiation: mean 25 g
ADMINISTRATION: oral gavage
- Doses: 1000 / 1500 / 2000 / 2500 / 3000 / 4000 mg/kg
- Volume administered or concentration: 1 ml / 100 g b.w.
- Post dose observation period: 14 days

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(27)

Type : LD50
Value : = 3450 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals : 5
Vehicle : other: corn oil
Doses :
Method : other: Acute Oral Toxicity
Year : 1964
GLP : no

Test substance	:	other TS: Origin: Esso Research and Engineering Company, 6 May 1964
Remark	:	Data were not suitable for statistical analysis, therefore the LD50-value was estimated by the authors.
Result	:	- Number of deaths at each dose, time of death: 34.6, 120, 417, 1,450 mg/kg: no deaths 5,000 mg/kg: 4 deaths after 4 (1), 24 (1) and 48 (2) hours 10,000 mg/kg: 5 deaths after 1 hour CLINICAL SIGNS: 34.6, 120, 417 mg/kg: none 1,450 mg/kg: depression, ptosis, lacrimation, labored respiration, and evidence of excessive urination; recovery was complete by the second day 5,000 mg/kg: depression, ptosis, lacrimation, masticatory movements, labored respiration, ataxia, absence of righting and placement reflexes, evidence of excessive urination, prostration, and death. Survivors completely recovered by the eighth day. 10,000 mg/kg: lacrimation, salivation, coma, and death NECROPSY FINDINGS: 34.6, 120, 417, 1,450 mg/kg: none 5,000 mg/kg: congestion of the lungs, kidneys, adrenals, and pancreas; gastrointestinal inflammation at death; none following sacrifice 10,000 mg/kg: congestion of the lungs and kidneys POTENTIAL TARGET ORGANS:
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 200-250 g - Controls: no ADMINISTRATION: - Doses: 34.6; 120; 417; 1,450; 5,000; 10,000 mg/kg bw administered orally by stomach tube animals fasted for only 3-4 hours before dosing - Volume administered or concentration: pure / 10 % / 1 % - Post dose observation period: 14 days; observations immediately, at 1, 4, and 24 hours, thereafter daily
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(33)

Type	:	LD50
Value	:	= 1500 mg/kg bw
Species	:	rat
Strain	:	other: breeder Voss
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: Emulsion 0.5 g carboxymethyl cellulose ad 100 ml dist. water
Doses	:	
Method	:	other
Year	:	1968
GLP	:	no
Test substance	:	no data
Remark	:	Study performed with animals of relatively low body weight.
Result	:	MORTALITY:

- Time of death: 1 hour to 3 days after dosing
- Number of deaths at each dose:
500 mg/kg: 0/10
1,000 mg/kg: 1/10
1,250 mg/kg: 1/10
1,500 mg/kg: 5/10
1,750 mg/kg: 7/10
2,000 mg/kg: 10/10
2,500 mg/kg: 8/10
LD50 confidence limits: 1,400-1,800 mg/kg

CLINICAL SIGNS:
> 1,000 mg/kg: general apathy, lateral position, irregular respiration

NECROPSY FINDINGS:
Results of animals that died
- increased secretion in stomach and small intestine
- thickening and hermorrhagic erosions of proventiculus lining
- urine retention
- hyperemia of liver
- pulmonary emphysema, edema or hyperemia
- splenic enlargement

Test condition : TEST ORGANISMS:
- Source: Voss
- Weight at study initiation: 80-115 g
- Controls: no
ADMINISTRATION:
- Doses: 0.5; 1.0; 1.25; 1.50; 1.75; 2.0; 2.5 g/kg bw
- Post dose observation period: 13 days
EXAMINATIONS:
gross examination of: coat of fur, skin, eye and conjunctiva, nose, mouth, ear, anus, preputial opening, vulva, subcutaneous connective tissue, abdominal cavity, pelvic cavity, peritoneum, esophagus, stomach, small intestine, large intestine, mesenteric lymph nodes, liver, pancreas, spleen, kidneys, urinary bladder, seminal vesicle, prostate, testicles, epididymis, ovary, uterus, vagina, thoracic cavity, pleura, heart, lungs, trachea, thymus gland, cerebrum, middle ear, application sites
STATISTICAL METHODS: Probit analysis

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(44)

Type : LD50
Value : = 1870 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: no data
Year : 1956
GLP : no
Test substance : no data

Remark	:	These data were provided by DUPONT in a letter to EPA. There were no further details given.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(29) (139)

Type	:	LD50
Value	:	= 2144 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	female
Number of animals	:	5
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1970
GLP	:	no
Test substance	:	other TS: commercial grade
Remark	:	Method published in 1969, result published in 1970
Test condition	:	TEST ORGANISMS: - Source: inhouse breeding colony - Weight at study initiation: 90-120 g - Controls: not reported ADMINISTRATION: - Doses: differing by a geometric factor of 2.0 - Volume administered or concentration: undiluted - Post dose observation period: 14 days
Reliability	:	(4) not assignable Documentation insufficient for assessment

(119) (120)

Type	:	LD50
Value	:	= 2370 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1976
GLP	:	no
Test substance	:	no data
Reliability	:	(3) invalid Original reference in Russian

(13) (139)

Type	:	LD50
Value	:	> 3200 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: no data
Year	:	1967
GLP	:	no
Test substance	:	no data

Remark : These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Reliability : (4) not assignable
Documentation insufficient for assessment

(30)

Type : LD50
Value : = 2000 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1976
GLP : no
Test substance : no data

Reliability : (3) invalid
Original reference in Russian

(13) (139)

Type : LD50
Value : = 3200 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: no data
Year : 1967
GLP : no
Test substance : no data

Remark : These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Reliability : (4) not assignable
Documentation insufficient for assessment

(30)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : = 7000 mg/m³
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle :
Doses :
Exposure time : 4 hour(s)
Method : other: Acute Inhalation Toxicity: Whole body exposure
Year : 1965
GLP : no
Test substance : other TS: Origin: Esso Research and Engineering Company, 2 Dec 1964,

Result	:	MORTALITY: - Number of deaths at each dose, time of death: 5000 mg/m3: 4 hours = 0; 14 days = 1 death 7000 mg/m3: 4 hours = 0; 14 days = 4 deaths >= 10000 mg/m3: 4 hours = 10 deaths LD50 confidence intervals: 5700-8600 mg/m3; slope function 1.27 CLINICAL SIGNS: 5000 mg/m3: none higher doses: ataxia and coma, dyspnea, piloerection, depression, decreased activity NECROPSY FINDINGS: >= 10000 mg/m3: - pulmonary congestion
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 200-225 grams - Number of animals: 10 - Controls: yes ADMINISTRATION: - Type of exposure: vapor - Concentrations: 5; 7; 10; 17.8 mg/l EXAMINATIONS: - Observations for deaths and toxic signs: 30 min intervals - post observation: 14 days, daily - gross pathology: at end of post observation period: all surviving animals
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(35)

Type	:	LC0
Value	:	>= 3500 mg/m³
Species	:	rat
Strain	:	Wistar
Sex	:	female
Number of animals	:	10
Vehicle	:	
Doses	:	
Exposure time	:	6 hour(s)
Method	:	other: Acute Inhalation Toxicity; Whole body exposure
Year	:	1964
GLP	:	no
Test substance	:	no data
Result	:	Deaths: None Clinical signs: Slight ptosis and lacrimation Autopsy: slight congestion of the lungs (after post-exposure the lungs of the surviving animals appeared normal).
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 275-300 grams - Number of animals: 10 - Controls: yes ADMINISTRATION: - Type of exposure: vapor - Concentrations: 3.5 mg/l EXAMINATIONS: - Observations for deaths and toxic signs: 15 min intervals - gross pathology: immediately following exposure: 2 animals/group incl.

control
at end of post observation period: all surviving animals
- post observation: 14 days, daily

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(33)

Type : LC0
Value : >= 3500 mg/m³
Species : mouse
Strain : Swiss
Sex : female
Number of animals : 10
Vehicle :
Doses :
Exposure time : 6 hour(s)
Method : other: Acute Inhalation Toxicity; Whole body exposure
Year : 1964
GLP : no
Test substance : no data

Result : Deaths: None
Clinical signs: Slight ptosis and lacrimation
Autopsy: slight congestion of the lungs (after post-exposure the lungs of the surviving animals appeared normal).

Test condition : TEST ORGANISMS:
- Weight at study initiation: 25-28 grams
- Number of animals: 10
- Controls: yes
ADMINISTRATION:
- Type of exposure: vapor
- Concentrations: 3.5 mg/l
EXAMINATIONS:
- Observations for deaths and toxic signs: 15 min intervals
- gross pathology:
immediately following exposure: 2 animals/group incl. control
at end of post observation period: all surviving animals
- post observation: 14 days, daily

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(33)

Type : LC0
Value : >= 3500 mg/m³
Species : guinea pig
Strain : Hartley
Sex : female
Number of animals : 10
Vehicle :
Doses :
Exposure time : 6 hour(s)
Method : other: Acute Inhalation Toxicity; Whole body exposure
Year : 1964
GLP : no
Test substance : no data

Result	:	Deaths: None Clinical signs: Slight ptosis and lacrimation Autopsy: no findings
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 400-425 grams - Number of animals: 10 - Controls: yes ADMINISTRATION: - Type of exposure: vapor - Concentrations: 3.5 mg/l EXAMINATIONS: - Observations for deaths and toxic signs: 15 min intervals - gross pathology: immediately following exposure: 2 animals/group incl. control at end of post observation period: all surviving animals - post observation: 14 days, daily
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(33)

Type	:	other: LT (Time to death)
Value	:	10572 mg/m³
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	
Method	:	other
Year	:	1940
GLP	:	no
Test substance	:	other TS: commercial grade, 10 % boiling below 212 degree C, 98 %
Remark	:	A vapor pressure of 0.51 hPa at 20 degree C corresponds to 503 ppm = 2,890 mg/m3 at saturation. The maximum reported here is 26,000 mg/m3. Probably the reported concentrations correspond mainly to volatile impurities. However at concentrations higher than 500 ppm, some of the material must have been in the aerosol form.
Result	:	MORTALITY: - A few rats died after 4 hours exposure >= 1840 ppm (10572 mg/m3). Causes of death are probably severe lung irritations. CLINICAL SIGNS: >= 800 ppm - Irritation of eyes and nose - lacrimation - swelling of nose - instability espiratory difficulty or irregularity - marked increase of intestinal peristalsis - light narcosis - some cases of low body temperature. - Exposure of >= 12 hours resulted in increase of heart rate, tremors, paralysis, uncoordinated scratching. Urine - Traces of albumin found

Surviving animals recovered quickly after end of exposure.

NECROPSY FINDINGS:
Results are given only for animals died from exposure to 1840 ppm
- Petechial and massive hemorrhages of the lungs, congestion of the stomach and liver, excess peritoneal fluid, a pale brownish color of the kidneys, orange tinted spleens.
- Reversible drop in red blood cells and hemoglobin.

2 h at 1340 ppm
- One case of early liver necrosis.
POTENTIAL TARGET ORGANS:
Organs affected in histopathological examinations: lung, liver and kidney.

Test condition : TEST ORGANISMS: rats
- Number of animals: ca. 200
- Controls: untreated
ADMINISTRATION:
- Type of exposure: inhalation
- Concentrations: A saturated atmosphere was generated by prolonged recirculation of air bubbled through isophorone (at max. 23 degree C) and diluted with pure air as required.
- Exposure levels: 300, 750, 880, 1370, 1840, 4600 ppm; 4600 ppm corresponds to 26000 mg/m3
- Exposure Time: between 6 and 24 hours
EXAMINATIONS:
21 sets of tissues were examined by histopathology, obviously the higher dose levels and 18 blood counts followed. Organs mentioned are lungs, liver, kidney, heart, spleen. Urine was also examined.

Reliability : (2) valid with restrictions
Study well documented acceptable for assessment.
Restriction: See remark

Flag : Critical study for SIDS endpoint
(110) (117) (135)

Type : LC0
Value : >= 26000 mg/m³
Species : guinea pig
Strain : no data
Sex : no data
Number of animals :
Vehicle :
Doses :
Exposure time :
Method : other: Acute Inhalation Toxicity; Whole body exposure
Year : 1940
GLP : no
Test substance : other TS: commercial grade, 10 % boiling below 212 degree C, 98 %

Remark : A vapor pressure of 0.51 hPa at 20 degree C corresponds to 503 ppm = 2,890 mg/m3 at saturation. The maximum reported here is 26,000 mg/m3. Probably the reported concentrations correspond mainly to volatile impurities. However at concentrations higher than 500 ppm, some of the material must have been in the aerosol form.

Result : MORTALITY:
- No guinea pig died.
CLINICAL SIGNS:
>= 800 ppm

- Irritation of eyes and nose
- lacrimation
- swelling of nose
- instability
espiratory difficulty or irregularity
- marked increase of intestinal peristalsis
- light narcosis
- some cases of low body temperature.
>= 840 ppm
- opacity of the cornea
- corneal necrosis
- Exposure of >= 12 hours resulted in increase of heart rate, tremors, paralysis, uncoordinated scratching.

Surviving animals recovered quickly after end of exposure.
NECROPSY FINDINGS:
No irreversible injuries. Findings were congested lungs, secondary pneumoniam, cloudy swelling, dilation, granular detritis and hyaline casts in kidneys

Test condition : TEST ORGANISMS: guinea pigs
- Number of animals: ca. 200
- Controls: untreated
ADMINISTRATION:
- Type of exposure: inhalation
- Concentrations: A saturated atmosphere was generated by prolonged recirculation of air bubbled through isophorone (at max. 23 degree C) and diluted with pure air as required.
- Exposure levels: 300, 750, 880, 1370, 1840, 4600 ppm; 4600 ppm corresponds to 26000 mg/m3
- Exposure Time: between 6 and 24 hours
EXAMINATIONS:
21 sets of tissues were examined and 18 blood counts followed. Organs mentioned are lungs, liver, kidney, heart, spleen. Urine was also examined.

Reliability : (2) valid with restrictions
Study well documented acceptable for assessment.
Restriction: See remark

Flag : Critical study for SIDS endpoint

(110) (117) (135)

Type : other: LC10
Value : = 40200 mg/m³
Species : rat
Strain : Wistar
Sex : male/female
Number of animals : 10
Vehicle : other: no vehicle
Doses :
Exposure time : 5 hour(s)
Method : other: described in reference
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Result : MORTALITY:
- Number of deaths at each dose:
no deaths at saturation at 20 degree C (2.3 mg/l)
10 % mortality at 40.2 mg/l (corresponds to 7000 ppm)
CLINICAL SIGNS: irritation of eyes and nose, accelerated breathing, narcosis; recovery of surviving animals within 12 hours without further symptoms

NECROPSY FINDINGS: bleeding of lung, other minor effects on respiratory tract
POTENTIAL TARGET ORGANS: lung

Test condition : TEST ORGANISMS:
- Weight at study initiation: ca. 200 g
- Controls: no
ADMINISTRATION:
- Type of exposure: air saturated at 20 degree C or higher temperatures was passed through the cages for five hours followed by isophorone free air for another hour.
- Concentrations: 400-7000 ppm = 2.3-40.2 mg/l
EXAMINATIONS: symptoms, mortality, post-observation period two weeks, autopsy

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(27)

Type : other: LC30
Value : = 40200 mg/m³
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals : 6
Vehicle : other: no vehicle
Doses :
Exposure time : 5 hour(s)
Method : other: described in reference
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Result : MORTALITY:
- Number of deaths at each dose:
no deaths at saturation at 20 degree C (2.3 mg/l)
30 % mortality at 40.2 mg/l (corresponds to 7000 ppm)
CLINICAL SIGNS: irritation of eyes and nose, accelerated breathing, narcosis; recovery of surviving animals within 12 hours without further symptoms
NECROPSY FINDINGS: bleeding of lung, other minor effects on respiratory tract
POTENTIAL TARGET ORGANS: lung

Test condition : TEST ORGANISMS:
- Weight at study initiation: ca. 2.5 kg
- Controls: no
ADMINISTRATION:
- Type of exposure: air saturated at 20 degree C or higher temperatures was passed through the cages for five hours followed by isophorone free air for another hour.
- Concentrations: 400-7000 ppm = 2.3-40.2 mg/l
EXAMINATIONS: symptoms, mortality, post-observation period two weeks, autopsy

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(27)

Type : LCLo
Value : ca. 3450 mg/m³
Species : rat
Strain :

Sex	:	
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	other: Acute Inhalation Toxicity
Year	:	
GLP	:	no
Test substance	:	no data
Result	:	single eight hour inhalation exposure to air saturated with isophorone (calculated concentration approximately 600 ppm) killed one of six rats.
Reliability	:	(4) not assignable Documentation insufficient for assessment (138) (139)
Type	:	other
Value	:	
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	other: Acute Inhalation Toxicity
Year	:	1956
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by DUPONT in a letter to EPA. There were no further details given.
Result	:	Breathing vapours in a state of saturation in air killed one of six animals in 8 h of exposure. Breathing a concentration of 5060 mg/m3 (880 ppm) for 1 h caused serious organ damage. No further information available.
Reliability	:	(4) not assignable Documentation insufficient for assessment (29)
Type	:	other
Value	:	
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	6 hour(s)
Method	:	other
Year	:	1967
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Result	:	Rats exposed to atmospheric concentrations of 2070 mg/m3 (360 ppm, calculated) for 6 hours produced symptoms of irritation but no permanent damage was evidenced.

Reliability : (4) not assignable
Documentation insufficient for assessment

(30)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	= 1700 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: 0.5 g carboxymethyl cellulose ad 100 ml. dist. water
Doses	:	
Method	:	other
Year	:	1968
GLP	:	no
Test substance	:	no data

Result :
MORTALITY:
- Time of death: 3 hours to 4 days after dosing
- Number of deaths at each dose:
1,120 mg/kg: 1/10
1,410 mg/kg: 4/10
1,840 mg/kg: 4/10
2,130 mg/kg: 8/10
3,400 mg/kg: 10/10
LD50 confidence limits: 1,450-2,000 mg/kg

CLINICAL SIGNS:
immediately after dosing general apathy, later on occasionally coma, cachexia, tremor, lacrimal secretion, reddening of treated areas. Doses at which clinical signs appeared are not defined)

NECROPSY FINDINGS:
Results of animals that died
- hyperemia and bleedings of subcutis (application area)
- uniform thickening of cutaneous gastric mucosa
- urine retention
- pulmonary emphysema, edema or hyperemia

Test condition : TEST ORGANISMS:
- Source: Gassner and Voss
- Weight at study initiation: 90-150 g
- Number of animals: 5 per dose and sex
- Controls: no
ADMINISTRATION:
- Type of exposure: dermal
- Concentrations: 50 g in 100 ml / 75 g in 100 ml / undiluted
EXAMINATIONS:
- observation period: 6-7 days
- gross pathology of: coat of fur, skin, eye and conjunctiva, nose, mouth, ear, anus, preputial opening, vulva, subcutaneous connective tissue, abdominal cavity, pelvic cavity, peritoneum, esophagus, stomach, small intestine, large intestine, mesenteric lymph nodes, liver, pancreas, spleen, kidneys, urinary bladder, seminal vesicle, prostate, testicles, epididymis, ovary, uterus, vagina, thoracic cavity, pleura, heart, lungs, trachea, thymus gland, cerebrum, middle ear, application sites

STATISTICAL METHODS: Probit analysis

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(45)

Type : LD50
Value : > 3160 mg/kg bw
Species : rabbit
Strain :
Sex : male/female
Number of animals : 4
Vehicle :
Doses :
Method : other: Acute Dermal Toxicity; Semi-Occlusive test
Year : 1964
GLP : no
Test substance : no data

Result : MORTALITY: none

CLINICAL SIGNS:
one animal at 3,160 mg/kg showed marked depression, labored respiration, sprawling, and depressed reflexes at the four-hour observation interval. The remaining animals seemed normal in appearance and behavior throughout the study and showed normal body weight gains.

NECROPSY FINDINGS:
no gross pathologic findings in any animal

Test condition : TEST ORGANISMS:
- Weight at study initiation: 2.1 - 2.8 kg
- Controls: none

ADMINISTRATION:
- Area covered: closely clipped, intact abdominal skin
- Occlusion: gauze and adhesive tape (occlusive conditions)
- Vehicle: none; undiluted substance
- Doses: 50, 200, 794, and 3,160 mg/kg
- Removal of test substance: after 24 hours, sponging with tap water or corn oil to remove any sample residue
- Post dose observation period:
  14 days; observations immediately, at 1, 4, and 24 hours, thereafter daily

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(33)

Type : LD50
Value : = 1200 mg/kg bw
Species : rabbit
Strain :
Sex : male/female
Number of animals : 6
Vehicle :
Doses :
Method : other: based on Draize, modified
Year : 1976
GLP : no data

Test substance	:	other TS: Elf Atochem S.A.
Result	:	MORTALITY: - Number of deaths and time at each dose: 9.2 g dose(0.6 +/- 0.4 g/kg absorbed): none 13.85 g dose (0.75 +/- 0.5 g/kg): 2 after 6 and 12 hours, resp. 23 g dose (1.7 +/- 0.9 g/kg): 3 after 3, 4, and 5 hours, resp. 23 g dose (2.85 +/- 1.3 g/kg): 3 after 4, 6, and 48 hours, resp. 32.3 g dose (2.5 +/- 1 g/kg): 6 after 2.5, 4x4, 5 hours, resp. CLINICAL SIGNS: accelerated breathing, prostration, narcosis, death (mostly within 6 hours) or recovery. The intensity of the erythema varied between animals. Recovery of the skin was not always complete within the postexposure period. Doses at which clinical signs appeared are not defined. POTENTIAL TARGET ORGANS: none identified (except skin) SEX-SPECIFIC DIFFERENCES: none identified
Test condition	:	TEST ORGANISMS: - Weight at study initiation: ca. 2.5 kg - Controls: yes ADMINISTRATION: - Occlusion: yes - Vehicle: none - Doses: 9.2; 13.85; 23; 23; 32.3 g absorbed: see Results - Removal of test substance: after 24 hours, unresorbed quantity determined EXAMINATIONS: mortality, clinical signs, skin reaction, 14 days postexposure observation, autopsy
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(27)

Type	:	LD50
Value	:	= 1380 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Dermal Toxicity
Year	:	1956
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by DUPONT in a letter to EPA. There were no further details given.
Result	:	Result reported as 1.50 ml/kg bw
Reliability	:	(4) not assignable Documentation insufficient for assessment

(29)

Type	:	LD50
Value	:	= 1390 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	

Vehicle	:	
Doses	:	
Method	:	other: Skin penetration LD50
Year	:	
GLP	:	no
Test substance	:	no data
Test condition	:	24-hour covered skin contact, no further details reported
Reliability	:	(4) not assignable Documentation insufficient for assessment

(138) (139)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	= 400 - 800 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: no data
Year	:	1967
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(30)

Type	:	LD50
Value	:	= 400 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: no data
Year	:	1967
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(30)

Type	:	other: LD20
Value	:	= 496.8 mg/kg bw
Species	:	mouse

Strain	:	CD-1
Sex	:	male/female
Number of animals	:	5
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: Acute i.p. Toxicity
Year	:	1988
GLP	:	no data
Test substance	:	no data
Reliability	:	(4) not assignable Documentation insufficient for assessment

(99)

Type	:	other: see Method freetext
Value	:	
Species	:	rat
Strain	:	
Sex	:	male
Number of animals	:	5
Vehicle	:	
Doses	:	
Route of admin.	:	other: Aspiration
Exposure time	:	
Method	:	other: Acute Aspiration Toxicity
Year	:	1963
GLP	:	no
Test substance	:	other TS: origin: Esso Research and Engineering Company
Method	:	Rats were anesthetized with diethyl ether vapor to the point of apnea and 0.2 ml of the test substance was placed in the mouth of each animal. Animals were held in a vertical position, and nostrils were closed to promote entry of liquid into the trachea. Then animals were returned to their cages for a 24 hour observation period, after which all surviving animals were sacrificed, and lungs were removed from all of the dosed animals.
Result	:	MORTALITY: - Time of death: within minutes after dosing - Number of deaths at each dose: 3 out of 5 The weight of the lungs was < 1.5 g for both of the two surviving animals and > 2.5 g for each of the three dead animals.
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 185; 190; 196; 205; 206 g - Controls: negative: tap water; positive: kerosene ADMINISTRATION: see Method freetext - Total volume applied: 0.2 ml (undiluted) - Removal of test substance: no EXAMINATIONS: mortality; weight of lung
Conclusion	:	Deaths were due to cardiac failure or respiratory arrest or both, rather than pulmonary edema. Isophorone presents potential aspiration hazard.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

(41)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	
Exposure time	:	4 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year	:	1981
GLP	:	no data
Test substance	:	no data

Result : No irritation was observed after occlusive and semi-occlusive application.

Test condition :
TEST ANIMALS:
- Strain: New Zealand
- Sex: male / female
- Source: various breeders
- Weight at study initiation: 2-4 kg
- Number of animals: 6 (male plus female)

ADMINISTRATION/EXPOSURE
- Preparation of test substance: none
- Area of exposure: laterally; shorn 15-24 hours in advance; 3 cm x 3 cm gauze pads with test substance
- Two patches were applied (one occlusive, one semi-occlusive):
  air-permeable circular bandage (semi-occlusive)
  air-tight plastic foil (occlusive)
- Total volume applied: 0.5 ml per patch (corresponds to ca. 1500 mg/kg bw)
- Postexposure period: 7 days
- Removal of test substance:
  each after 4 hours; rinsed with water and dried

EXAMINATIONS
- Scoring system: 5th Amendment to 67/548/EEC (1983)
- Examination time points:
  1, 24, 48, 72 hours, and 7 days after removal

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

(105)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	4
Vehicle	:	
PDII	:	
Result	:	irritating
Classification	:	
Method	:	other: 24 h-Occlusive-Test
Year	:	1964
GLP	:	no
Test substance	:	other TS

Remark	:	The study was part of an acute dermal toxicity study.
Result	:	50 mg/kg b.w.: no irritation 200 mg/kg b.w.: slight erythema and desquamation during the first four days 794 mg/kg b.w.: slight edema, atonia and slight to moderate erythema during the first two days; slight desquamation from the second to the last day of the observation period 3160 mg/kg b.w.: slight edema at the first day; slight atonia from the second to the 10th day; slight to moderate erythema during the first five days; slight to moderate desquamation from the second day to the last day of the observation peri
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 2.1 - 2.8 kg - Controls: none ADMINISTRATION: - Area covered: closely clipped, intact abdominal skin - Occlusion: dental dam binder - Vehicle: none; undiluted substance - Doses: 50, 200, 794, and 3,160 mg/kg (200 mg/kg bw isophorone corresponds to about 0,5 ml undiluted substance) - Removal of test substance: after 24 hours, sponging with tap water or corn oil to remove any sample residue - Post dose observation period: 14 days; observations immediately, at 1, 4, and 24 hours, thereafter daily
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: Exposure time was 24 hours.
Flag	:	Critical study for SIDS endpoint

(33)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: Draize Test according to the US Food and Drug Administration, Federal Register No. 191-11
Year	:	1972
GLP	:	no
Test substance	:	no data
Result	:	AVERAGE SCORE - Erythema: 0.4 (intact skin: 0.25/4; abraded skin 0.58/4) - Edema: 0 REVERSIBILITY: yes
Test condition	:	TEST ANIMALS: - Strain: Fauve de Bourgogne - Weight at study initiation: 2 - 2.5 kg ADMINISTRATION/EXPOSURE - Vehicle: none - Total volume applied: 0.5 ml

- Postexposure period: 48 hours
- Removal of test substance: no
- Application to both intact and scarified skin
EXAMINATIONS
- Scoring system: based on Draize
- Examination time points: 24 and 72 hours

Reliability : (1) valid without restriction
Test procedure in accordance with national standard methods
Flag : Critical study for SIDS endpoint

(27) (137)

Species	:	guinea pig
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	moderately irritating
Classification	:	
Method	:	other: no data
Year	:	1967
GLP	:	no
Test substance	:	no data

Remark : These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Result : The test gave no evidence that the compound was absorbed through the skin.
Reliability : (4) not assignable
Documentation insufficient for assessment

(30)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	1.3
Result	:	slightly irritating
Classification	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	no data

Method : U.S. Consumer Product Safety Commission, Code of Federal Regulations, Title 16, Section 1500.41;
Evaluation with Draize scores
Test condition : ADMINISTRATION:
- Volume administered or concentration: 0.5 ml
- Post dose observation period: 48 hours
- Application to both intact and scarified skin
EXAMINATIONS: 24 and 72 hours
Reliability : (1) valid without restriction
Test procedure in accordance with national standard methods

(57)

Species	:	rabbit
Concentration	:	undiluted

Exposure	:	
Exposure time	:	1 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other
Year	:	1981
GLP	:	no data
Test substance	:	no data
Remark	:	These results are part of an investigation described earlier (Potokar, exposure time 4 hours)
Result	:	No irritation was observed after occlusive and semi-occlusive application.
Test condition	:	TEST ANIMALS: - Strain: New Zealand - Sex: male / female - Source: various breeders - Weight at study initiation: 2-4 kg - Number of animals: 6 (male plus female) ADMINISTRATION/EXPOSURE - Preparation of test substance: none - Area of exposure: laterally; shorn 15-24 hours in advance; 3 cm x 3 cm gauze pads with test substance - Two patches were applied (one occlusive, one semi-occlusive): air-permeable circular bandage (semi-occlusive) air-tight plastic foil (occlusive) - Total volume applied: 0.5 ml per patch - Postexposure period: 7 days - Removal of test substance: each after 4 hours; rinsed with water and dried EXAMINATIONS - Scoring system: 5th Amendment to 67/548/EEC (1983) - Examination time points: 1, 24, 48, 72 hours, and 7 days after removal
Reliability	:	(2) valid with restrictions Guideline study with restriction: Exposure time was 1 hour.

(105)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	not rinsed
Number of animals	:	6
Vehicle	:	
Result	:	irritating
Classification	:	
Method	:	Draize Test
Year	:	1964
GLP	:	no
Test substance	:	other TS: Origin: Esso Research and Engineering Company, 6 May 1964
Result	:	Immediately following application of the test material, all

of the animals exhibited scrambling and preening; phonation was noted in one animal.

Irritation was moderate to severe and generally consisted of moderate or marked erythema, chemosis, and discharge, slight corneal opacity, and apparent corneal sloughing. One animal showed slight, transient iritis. The sodium fluorescein examination on the seventh day confirmed the presence of corneal lesions in two animals; re-examination at 14 days revealed no corneal damage.

Test condition : TEST ANIMALS:
- Sex: male/female
- Weight at study initiation: 2.2-2.7 kg
- Number of animals: 6 per dose group and sex
- Controls: untreated eye

ADMINISTRATION/EXPOSURE
- Vehicle: undiluted into conjunctival sac of left eye, closed for 30 seconds
- Postexposure period:
1, 4, 24 hours; 2, 3, 4, 7, 10, 14 days

EXAMINATIONS
- Scoring system: Draize, 1959

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(33)

Species : rabbit
Concentration : undiluted
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 6
Vehicle :
Result : irritating
Classification : irritating
Method : other: Draize Test according to the US Food and Drug Administration, Federal Register No. 191-11
Year : 1965
GLP : no
Test substance : no data

Result : AVERAGE SCORE
- Cornea: 14/80
- Iris: 0/10
- Conjuntivae: 6/20
- Overall irritation score: 20/110

REVERSIBILITY: yes

Test condition : EXAMINATIONS
- Ophtalmoscopic examination: after 24, 48, and 72 hours
- Scoring system: according to Draize
- Observation period: 72 hours
- Tool used to assess score: untreated eye served as control

Reliability : (1) valid without restriction
Test procedure in accordance with national standard methods

Flag : Critical study for SIDS endpoint

(27) (137)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	other: rinsed after 2 seconds / 4 seconds / not rinsed (3 animals each)
Number of animals	:	3
Vehicle	:	
Result	:	irritating
Classification	:	irritating
Method	:	Draize Test
Year	:	1959
GLP	:	no
Test substance	:	no data
Remark	:	Scores are averages of results after 24, 48, and 72 hours
Result	:	Group 1: not rinsed AVERAGE SCORE - Cornea: 15/80 - Iris: 0/10 - Conjuntivae: 7/20 - Overall irritation score: 22/110 Group 2: rinsed after 2 seconds with 20 ml warm water AVERAGE SCORE - Cornea: 21/80 - Iris: 0/10 - Conjuntivae: 6/20 - Overall irritation score: 27/110 Group 3: rinsed after 4 seconds with 20 ml warm water AVERAGE SCORE - Cornea: 12/80 - Iris: 0/10 - Conjuntivae: 5/20 - Overall irritation score: 17/110 REVERSIBILITY: yes
Test condition	:	TEST ANIMALS: - Strain: Fauve de Bourgogne - Controls: untreated eye EXAMINATIONS - Ophtalmoscopic examination: after 24, 48, and 72 hours, 4 days, 7 days, later until complete reversibility - Scoring system: Draize et al. (1944), J. Pharmacol. Exp. Ther. 82, 377-390 - Observation period: 72 hours and more
Reliability	:	(1) valid without restriction Comparable to guideline study
Flag	:	Critical study for SIDS endpoint

(27) (137)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	:	other: Eye Irritation

Year	:	1956
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by DUPONT in a letter to EPA. There were no further details given.
Result	:	One large drop produced severe surface burns in the rabbit eye. A small drop, as might occur in a fine spray, caused minor injury.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(29)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	irritating
Classification	:	
Method	:	other: Eye Irritation
Year	:	1946
GLP	:	no
Test substance	:	no data
Result	:	AVERAGE SCORE - Overall irritation score: 4
Test condition	:	TEST ANIMALS: - Strain: Albino, not specified ADMINISTRATION/EXPOSURE - Amount of substance instilled: 0.005 - 0.1 ml to centre of the cornea - Exposure period: 18 - 24 h EXAMINATIONS - Scoring system: maximum 10 scores - Tool used to assess score: eye examined in strong diffuse daylight, then stained with fluorescein
Reliability	:	(4) not assignable Documentation insufficient for assessment

(17)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	not rinsed
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	other
Year	:	
GLP	:	no
Test substance	:	no data
Method	:	U.S. Consumer Product Safety Commission, Code of Federal Regulations, Title 16, Section 1500.42; Evaluation with Draize scores

Test condition	:	Examination after 24, 48, 72 hours
Reliability	:	(1) valid without restriction Test procedure in accordance with national standard methods

(58)

Species	:	other: no data
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	moderately irritating
Classification	:	
Method	:	other: no data
Year	:	1967
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by Eastman Kodak Company in a letter to EPA. There were no further details given.
Result	:	Toxic signs: cornea damage Reversibility: yes (14 days)
Reliability	:	(4) not assignable Documentation insufficient for assessment

(30)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 10 % intracutaneous 2nd: Challenge undiluted occlusive epicutaneous 3rd: Challenge undiluted occlusive epicutaneous
Number of animals	:	20
Vehicle	:	other: corn oil
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	OECD Guide-line 406 "Skin Sensitization"
Year	:	1981
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	RESULTS OF TEST - Sensitization reaction: 0/20
Test condition	:	TEST ANIMALS: - Strain: Albino, Bor: DHPW - Sex: female - Source: F. Winkelmann, Borchen (DE) - Weight at study initiation: 314.2 g (mean) - Controls: 10 animals ADMINISTRATION/EXPOSURE - Concentration in Freuds Complete Adjuvant (FCA): 10 % - Positive control: no
Reliability	:	(2) valid with restrictions Guideline study with acceptable restrictions: no positive control group (not required by 1981 version of guideline)
Flag	:	Critical study for SIDS endpoint

21.07.2003

(60)

Type	:	other: no data
Species	:	human
Number of animals	:	10
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: no data
Year	:	1956
GLP	:	no
Test substance	:	no data
Remark	:	These data were provided by DUPONT in a letter to EPA. There were no further details given.
Test condition	:	Ten human volunteers were tested.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(29)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	16 days
Frequency of treatm.	:	5 days/week
Post exposure period	:	none
Doses	:	125, 250, 500, 1000, or 2000 mg/kg bw d
Control group	:	yes, concurrent vehicle
NOAEL	:	= 500 mg/kg bw
Method	:	other: Repeated Dose Toxicity, U.S. NTP
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water
Remark	:	Study was performed to select dose levels for the 13 week-study.
Result	:	NOAEL (NOEL), LOAEL (LOEL): NOAEL based on reduced body weight gain. - Mortality and time to death: 4 females that received 2000 mg/kg died (days 2, 2, 3, 3) 1 male that received 2000 mg/kg died (day 2) - Clinical signs: All dosed rats were lethargic after dosing. - Body weight gain: Body weights were lower by m, 1000 mg/kg: 13.9 % f, 1000 mg/kg: 6.7 % m, 2000 mg/kg: 25.2 % f, 2000 mg/kg: 11.4 % - Gross pathology: No compound-related pathologic effects were observed. - Histopathology:

No lesions were noted upon microscopic examination of the tissues from six selected rats from the two highest dose groups.

Test condition : TEST ORGANISMS
- Age: 47-54 days
- Number of animals: 5 per dose and sex
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 5 days/week, 12 doses on 16 days
- Vehicle: corn oil
- Total volume applied: 1 ml
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: twice daily
- Mortality: twice daily
- Body weight: days 0 and 16
- Hematology: no
- Urinalysis: no
- Biochemistry: no
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weight: no
- Macroscopic:
skin, mammary gland, mandibular lymph node, salivary gland, thigh muscle, sciatic nerve, vertebrae, costochondral junction (rib), thymus, larynx, lungs and bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejenum, eyes, ileum, colon, cecum, rectum, mesenteric lymph node, liver, pancreas, spleen, kidney, adrenal glands, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, nasal cavity, brain, pituitary gland, spinal cord.
- Microscopic:
(selected animals - 2000 mg/kg bw: 3m 1f; 100 mg/kg bw: 2f):
skin, mammary gland, sciatic nerve, salivary gland, mandibular lymph node, thymus, heart, lungs, trachea, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, mesenteric lymph node, pancreas, spleen, liver, kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate/testes or ovaries/uterus, brain, pituitary gland, bone marrow, spinal cord, and nasal cavity
STATISTICAL METHODS: survival, body weight, dose-related effects are statistically evaluated

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ weight determination, no clinical biochemistry

Flag : Critical study for SIDS endpoint

(12) (98)

Type : Sub-acute
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 16 days
Frequency of treatm. : 5 days/week
Post exposure period : none
Doses : 125, 250, 500, 1000, or 2000 mg/kg bw d
Control group : yes, concurrent vehicle
NOAEL : = 125 mg/kg bw

NOAEL (male mice)	:	= 500 mg/kg bw
Method	:	other: Repeated Dose Toxicity, U.S. NTP
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water
Remark	:	Study was performed to select dose levels for the 13 week-study.
Result	:	NOAEL (NOEL), LOAEL (LOEL): NOAEL based on reduced body weight gain - Mortality and time to death: all mice administered 2000 mg/kg died (time not reported) - Clinical signs: Male and female mice that received 1000 mg/kg staggered after dosing. - Body weight gain: Body weights were lower by m, 1000 mg/kg: 7.8 % f, >= 250 mg/kg: 7.3-9.3 % Male mice lost weight during week 1, probably as a consequence of fighting. - Gross pathology: No compound-related pathologic effects were observed. - Histopathology: No lesions were noted in tissues examined microscopially from two male and two female mice from the 1000 mg/kg dose group.
Test condition	:	TEST ORGANISMS - Age: 47-61 days - Number of animals: 5 per dose and sex ADMINISTRATION / EXPOSURE - Duration of test/exposure: 5 days/week, 12 doses on 16 days - Vehicle: corn oil - Total volume applied: 0.5 ml CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: twice daily - Mortality: twice daily - Body weight: days 0 and 16 - Hematology: no - Urinalysis: no - Biochemistry: no ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weight: no - Macroscopic: skin, mammary gland, mandibular lymph node, salivary gland, thigh muscle, sciatic nerve, vertebrae, femur, costochondral junction (rib), thymus, larynx, lungs and bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejenum, eyes, ileum, colon, cecum, rectum, mesenteric lymph node, liver, gallbladder, pancreas, spleen, kidney, adrenal glands, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, nasal cavity, brain, pituitary gland, spinal cord. - Microscopic: (selected animals - 1000 mg: 2m, 2f): skin, mammary gland,

sciatic nerve,
salivary gland, mandibular lymph node, thymus, heart, lungs, trachea, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, mesenteric lymph node, pancreas, spleen, liver, gallbladder, kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate/testes or ovaries/uterus, brain, pituitary gland, bone marrow, spinal cord, and nasal cavity
STATISTICAL METHODS: survival, body weight, dose-related effects are statistically evaluated

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ weight determination, no clinical biochemistry

Flag : Critical study for SIDS endpoint

(12) (98)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	5 days/week
Post exposure period	:	none
Doses	:	62.5, 125, 250, 500, or 1000 mg/kg bw d
Control group	:	yes, concurrent vehicle
NOAEL	:	= 500 mg/kg bw
Method	:	other: Repeated Dose Toxicity, U.S. NTP
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water

Result : NOAEL (NOEL), LOAEL (LOEL): NOAEL based on body weight
- Mortality and time to death: 1 female (1000 mg/kg, week 5)

- Clinical signs:
Rats that received 1000 mg/kg were sluggish and lethargic after dosing.

Body weight: 1000 mg/kg bw m: slightly reduced body weight gain

- Gross pathology:
No compound-related pathologic effects were observed.

- Histopathology:
No compound-related pathologic effects were observed.

- Other:
Toxic changes in the kidneys were not found, even by recuts and special stains.

Test condition : TEST ORGANISMS
- Age: 8 weeks
- Number of animals: 10 per dose and sex
ADMINISTRATION / EXPOSURE
- Vehicle: corn oil
- Total volume applied: 1 ml
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: twice daily
- Mortality: twice daily

- Body weight: weekly
- Hematology: no
- Urinalysis: no
- Biochemistry: no
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weight: no
- Macroscopic: no details reported
- Microscopic:
skin, mammary gland, sciatic nerve, salivary gland, mandibular lymph node, thymus, heart, lungs, trachea, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, mesenteric lymph node, pancreas, spleen, liver, kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate/testes or ovaries/uterus, brain, pituitary gland, bone marrow, spinal cord, and nasal cavity
STATISTICAL METHODS: survival, body weight, dose-related effects are statistically evaluated

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ weight determination, no clinical biochemistry

Flag : Critical study for SIDS endpoint

04.07.2003 (12) (98)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	other: CFE
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	750, 1500 and 3000 ppm diet: males 57.0, 102.5 and 233.8 mg/kg bw d; females 78.9, 163.8 and 311.8 mg/kg bw d
Control group	:	yes, concurrent no treatment
NOAEL	:	= 102.5 mg/kg bw
NOAEL (female rats)	:	>= 311.8 mg/kg bw
Method	:	other: Repeated Dose Toxicity
Year	:	1972
GLP	:	no
Test substance	:	other TS: Origin: International Chemical Corp., New York, 11 Aug 1971

Result : NOAEL: m: 1500 ppm diet (reduced body weight gain), equivalent to 102.5 mg/kg bw; f: 3000 ppm diet, equivalent to 311.8 mg/kg bw.
ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX
mean daily compound consumption (data given in study):
- males 57.0, 102.5 and 233.8 mg/kg bw d
- females 78.9, 163.8 and 311.8 mg/kg bw d
TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: 1 m (control group), 1 f (3000 ppm) - deaths were due to intercurrent infection
- Clinical signs: none
- Body weight gain: 3000 ppm m: significant reduced body weight gain ($P < 0.01$); further observed changes returned to normal in the subsequent weeks
- Food/water consumption: no evidence of refusal
- Clinical chemistry: all values within normal limits
- Haematology: all values within normal limits

- Urinalysis: all values comparable to controls
- Organ weights:
kidney, testes m: slightly increased mean organ to body weight ratio (considered not compound related by the authors; not statistically significant)
- Gross pathology: All viscera were normal in appearance and color, no lesions were observed
- Histopathology: no evidence of significant pathology

Test condition : TEST ORGANISMS
- Number of animals: 20 per dose and sex
- After 4 weeks, 5 animals per sex and dose group were killed for blood analysis
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: daily
- Mortality: daily
- Body weight: weekly
- Food consumption: weekly
- Water consumption: weekly
- Ophthalmoscopic examination: none
- Hematology: after 4 weeks and at end of study:
determination of hemoglobin, hematocrit, erythrocyte counts, leukocyte counts, and differential leukocyte determinations
- Biochemistry: after 4 weeks and at end of study: blood glucose, blood urea nitrogen, serum glutamic oxaloacetic transaminase, serum alkaline phosphatase, total serum protein, total serum bilirubin, serum albumin, lactic acid dehydrogenase, cholesterol, calcium, phosphate, and uric aid
- Urinalysis: after 4 weeks and at end of study: pH, glucose, ketones, albumin, occult blood, microscopic examination of sediment
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weights: organ-body weight ratios: heart, liver, kidney, adrenals, thyroid, brain, testes
- Macroscopic:
after 4 weeks and at study termination: lungs, heart, intestines, kidneys, spleen, liver, urinary bladder;
- weights: heart, liver, kidney, adrenals, thyroid, brain, testes (males) for 10 males and 10 females of each dose level
- Microscopic:
at study termination only: 5 males and 5 females each from high dose and control groups: brain, pituitary, eye, thyroid, lung, heart, liver, kidney, adrenals, urinary bladder, mediastinal lymph node, pancreas, spleen, colon, bone marrow, skeletal muscle, testes and prostate (male), ovary and uterus (female)
5 males and 5 females each from medium and low dose groups: liver, kidney
STATISTICAL METHODS: All data were evaluated statistically.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(108) (138)

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 13 weeks

Frequency of treatm.	:	5 days/week
Post exposure period	:	none
Doses	:	62.5, 125, 250, 500, or 1000 mg/kg bw d
Control group	:	yes, concurrent vehicle
NOAEL	:	= 500 mg/kg bw
NOAEL (male mice)	:	= 1000 mg/kg bw
Method	:	other: Repeated Dose Toxicity, U.S. NTP
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water
Result	:	NOAEL (NOEL), LOAEL (LOEL): NOAEL based on mortality - Mortality and time to death: 3 females that received 1000 mg/kg died (weeks 8, 11, 13) - compound related 1 female and 3 males died accidentally or were missing - Body weight gain: no dose related effect - Gross pathology: No compound-related pathologic effects were observed. - Histopathology: No compound-related pathologic effects were observed. - Other: The kidneys of high dose and vehicle control male and female mice were reviewed on two separate occasions to confirm a lack of evidence of nephrotoxicity.
Test condition	:	TEST ORGANISMS - Age: 8 weeks - Number of animals: 10 per dose and sex ADMINISTRATION / EXPOSURE - Vehicle: corn oil - Total volume applied: 0.5 ml CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: twice daily - Mortality: twice daily - Body weight: weekly - Hematology: no - Urinalysis: no - Biochemistry: no ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weight: no - Macroscopic: no details reported - Microscopic: skin, mammary gland, sciatic nerve, salivary gland, mandibular lymph node, thymus, heart, lungs, trachea, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, mesenteric lymph node, pancreas, spleen, liver, gallbladder, kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate/testes or ovaries/uterus, brain, pituitary gland, bone marrow, spinal cord, and nasal cavity STATISTICAL METHODS: survival, body weight, dose-related effects are statistically evaluated
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ

weight determination, no clinical biochemistry

Flag : Critical study for SIDS endpoint

(12) (98)

Type	:	Sub-chronic
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	other: oral gelatine capsules
Exposure period	:	90 days
Frequency of treatm.	:	daily (7 days/week)
Post exposure period	:	none
Doses	:	35, 75 and 150 mg/kg bw d
Control group	:	other: yes, concurrent
NOAEL	:	>= 150 mg/kg bw
Method	:	other: Repeated Dose Toxicity
Year	:	1972
GLP	:	no
Test substance	:	other TS: Origin: International Chemical Corp., New York, 11 Aug 1971

Result :

NOAEL >= 150 mg/kg bw
TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: no mortalities
- Clinical signs: some incidences of soft stools in 75 and 150 mg/kg bw d groups
- Body weight gain: no significant effect
- Food consumption: within normal limits
- Clinical chemistry: all values within normal limits
- Haematology: all values within normal limits
- Urinalysis: all values within normal limits
- Organ weights: no significant differences in organ-body weight ratios
- Gross pathology: all organs normal in appearance and color
- Histopathology: All experimental tissues were within normal limits and were comparable to controls. There was no evidence of any definitive signs of celluar change.

Test condition :

TEST ORGANISMS
- Number of animals: 4 per dose and sex
ADMINISTRATION / EXPOSURE
- Type of exposure: oral, in gelatine capsules once daily
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: daily
- Mortality: daily
- Body weight: weekly
- Food consumption: daily
- Hematology: monthly: erythrocyte, leucocyte and differential leukocyte counts, hematocrit and hemoglobin determinations
- Biochemistry: monthly: blood glucose, blood urea nitrogen, serum glutamic oxoloacetic transaminase, serum alkaline phosphatase, total serum protein, total serum bilirubin, serum albumin lactic acid dehydrogenase, cholesterol, calcium, phosphate, uric acid
- Urinalysis: monthly: pH, occult blood, ketones, albumin and sugar, microscopic examination of sediment
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weight: Organ-body weight ratio determined: heart, liver, kidney, spleen, thyroid, adrenals, brain
- Macroscopic:
lungs, heart, intestines, kidneys, spleen, liver, urinary

bladder;
- weights: heart, liver, spleen, adrenals, thyroid, brain, kidneys
- Microscopic:
high dose and control groups:
brain, pituitary, eye, submaxillary gland, thyroid, heart, lung, liver, kidneys, adrenals, pancreas, spleen, mesentery lymph nodes, stomach, small intestine, colon, urinary bladder, bone marrow, muscle, sciatic nerve, spinal cord, skin. Males: testes, prostate, seminal vesicles; female: ovary, uterus, mammary gland.
medium and low dose groups:
liver, kidney
STATISTICAL METHODS: All data were evaluated statistically.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(109) (138)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : other: Charles River Caesarian-derived
Route of admin. : inhalation
Exposure period : 28 days
Frequency of treatm. : 6 hours/day; 5 days/week
Post exposure period : none
Doses : 208 +/- 10 mg/m3 air corresponds to about 36 ppm
Control group : yes, concurrent no treatment
Method : other: Repeated Dose Toxicity
Year : 1968
GLP : no
Test substance : other TS: Origin: Esso Research and Engineering Company, 31 March 1966, used as received

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: no mortalities
- Clinical signs: slight nasal bleeding on day 3, reddish-brown discoloration of the fur surrounding the nasal regions on days 6 through 8
- Body weight gain: body weight only of male rats was significantly reduced compared to control
- Haematology: differential blood count: increase of the percentage of lymphocytes (pre-exposure: 81.6 and 80.2 % among males and females, resp.; postexposure: 84.6 and 86.0 %, resp.), decrease in the percentage of segmented neutrophiles (pre-exposure: 17.4 and 18.2 % among males and females, resp.; postexposure: 14.6 and 13.3 %, resp.).
- Organ weights: absolute and relative liver weight only of male rats were significantly reduced compared to control
- Gross pathology: no clear-cut compound-related abnormalities
- Histopathology: no unequivocal change

Test condition : TEST ORGANISMS
- Number of animals: 10 males and 10 females
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 20 exposures of 6 hours each
- Type of exposure: inhalation
- Post exposure period: none
- Concentrations: nominal 250 mg/m3; daily analytical

determination (208 mg/m3) may be less precise than nominal due to incomplete recovery in analysis
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: at frequent intervals
- Mortality: at frequent intervals
- Body weight: at study initiation and termination
- Haematology: at study initiation and termination, identical 50 % of animals of each group
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: brain, pituitary, trachea, thyroid, parathyroid, lungs, heart, liver, spleen, stomach, small intestine, large intestine, adrenals, kidneys, urinary bladder, gonads, femur
- organ weights: lungs, liver, kidneys, adrenal, spleen
- Microscopic: lungs, liver, kidneys, adrenal, spleen for three males and three females of each group
STATISTICAL METHODS: performed on body and organ weights

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(37)

Type : Sub-acute
Species : mouse
Sex : male
Strain : other: Swiss OF1 (IFFA Credo)
Route of admin. : inhalation
Exposure period : 4, 9 or 14 days
Frequency of treatm. : 6 hours/day
Post exposure period : no
Doses : 164 +/- 21 mg/m3 (28 ppm) or 513 +/- 36 mg/m3 (90 ppm)
Control group : yes
Method : other
Year : 1995
GLP : no data
Test substance : other TS: Origin: Merck/Schuchardt, Darmstadt, Germany, Purity: 98 %

Remark : Study designed in order to investigate a special focus: irritative potential of isophorone.

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Clinical signs: none
- Histopathology: No histological abnormalities were observed in any location of the nasal passages, the trachea or the lungs.

Test condition : TEST ORGANISMS
- Weight at study initiation: 20-25 g
- Number of animals: 10 per dose group, 5 in control group
ADMINISTRATION / EXPOSURE
- Type of exposure: whole body
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: yes, no details reported
- Mortality: yes, no details reported
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: none
- Microscopic: trachea, lungs, nasal airways

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag	:	Critical study for SIDS endpoint

(150)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	inhalation
Exposure period	:	4 months (f), 6 months (m)
Frequency of treatm.	:	6 hours/day, 5 days/week
Post exposure period	:	no data
Doses	:	2873 mg/m3 (500 ppm)
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	other TS: Elf Atochem S.A.
Remark	:	Study was designed as a one-generation study (see chapter 5.8.1)
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: clinical signs: irritation of eyes and nose - Mortality: 1/10 of exposed females and 2/10 of exposed males died Lung Congestion of lungs (in controls and exposed animals) Liver granular state and clarification of liver cytoplasma (in controls and exposed animals)
Test condition	:	TEST ORGANISMS - Weight at study initiation: approximately 140 g - Number of animals: 10 males, 10 females ADMINISTRATION / EXPOSURE - Type of exposure: inhalation - Vehicle: air - Control group: 10 males, 10 females EXAMINATIONS Mortality Clinical signs: no data on frequency Body weight: no data on frequency Male rats: Autopsy of lungs, histological examinations on liver (no detailed data given)
Reliability	:	(2) valid with restrictions Regarding specific topics (investigation of the respiratory tract) the study is well documented.
Flag	:	Critical study for SIDS endpoint

(27)

Type	:	Sub-chronic
Species	:	other: rat and guinea pig
Sex	:	male/female
Strain	:	other: Wistar and not specified
Route of admin.	:	inhalation
Exposure period	:	6 weeks
Frequency of treatm.	:	8 hours/day; 5 days/week
Post exposure period	:	14 days
Doses	:	144, 287, 575, 1150 and 2874 mg/m3 air (25, 50, 100, 200, 500 ppm)
Control group	:	other: yes, concurrent
Method	:	other: Repeated Dose Inhalation
Year	:	1942

GLP	:	no
Test substance	:	other TS: commercial grade
Remark	:	In Rowe, Wolf (1962) it is reported, that the impurities in the used isophorone are probably to high to consider the results of this investigation.
Result	:	NOAEL (NOEL), LOAEL (LOEL): A NOAEL is not derivable due to possible impurities. TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: 100 ppm: 2 of 16; 200 ppm: 3 of 18; 500 ppm: 9 of 20. Times of death not reported. >= 50 ppm Kidney - pale or brown kidneys - Congestion, Dilation of Bowman's capsule; cloudy swelling of convoluted tubular epithelium (25 ppm: 0/10, 50 ppm: 4/6, 100 ppm: 6/8, 200 ppm: 4/7, 500 ppm: 6/9) >= 100 ppm Body weight: - poor growth Lungs: - Lungs were often irritated, resulting in congestion, capillary leakage and desquamation of epithelium. 500 ppm Clinical signs - Chronic conjunctivitis and nasal irritiation sometimes proceeding to bloody exudate Clinical chemistry - Blood cell changes - Urine - albumine Lung - Pulmonary inflammation Other Examinations - Liver, spleen and small intestine was sometimes congested; the findings were not dose-related - Ophthalmoscopic examination: No animals developed corneal necrosis Remark: No distinction was made between results with rats and guinea pigs. Animals differed only slightly, the rats being more susceptible to the test materials.
Test condition	:	TEST ORGANISMS - male Wistar rats; guinea pigs of mixed sex - Age: young, in the stage of active growth - Weight at study initiation: rats 90-120 g; guinea pigs 250-300 g - Number of animals: 10 rats and 10 guinea pigs per concentration ADMINISTRATION / EXPOSURE - Pre exposure period: 2 weeks CLINICAL OBSERVATIONS AND FREQUENCY: - Body weight: weekly - Haematology: "was followed" - Urinalysis: pooled, at least once ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND

MICROSCOPIC):
97 out of 170 exposed animals plus 12 control animals: Liver, kidney, spleen, lung, adrenal, heart muscle; some animals also voluntary muscle, pancreas, testicle, small intestine

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag : Critical study for SIDS endpoint

(110) (118)

Type : Chronic
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : inhalation
Exposure period : 18 months
Frequency of treatm. : 6 hours/day; 5 days/week
Post exposure period : none
Doses : 1436 mg/m3 (250 ppm)
Control group : yes, concurrent vehicle
Method : other: see Reference
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Remark : The report suggests that two identical groups of males and two groups of females were exposed in addition to the unexposed control groups, corresponding to a total of six groups. As the presentation of results does not refer to absolute numbers of animals but only to percentages of total animals, their misinterpretation can be excluded.

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: 40 % each of males and females, controls as well as exposed died; time not reported
- Signs of irritation: slight irritation on conjunctiva, no opacity of cornea, by end of third week bloody exsudate from nose indicating irritation of mucosa
- Body weight gain: no difference between exposed and control
- Haematology: no differences between exposed and control
- Urinalysis: no differences between exposed and control; pH approximately 9 in all groups
- Gross pathology: More or less pronounced hemorrhages of the lungs were observed in exposed and control animals likewise. Discoloration of the livers was also observed independent on exposure. The other organs inspected appeared normal.
- Histopathology: Lesions in lungs were found in both exposed and control animals. Microvacuolisation in livers was more pronounced in exposed animals than in controls. The other organs were normal, or changes were considered to be insignificant.

Test condition : TEST ORGANISMS
- Weight at study initiation: 200 g
- Number of animals:
10 males and 10 females each in exposed and control groups
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 18 months
- Type of exposure: inhalation
- Vehicle: air

CLINICAL OBSERVATIONS AND FREQUENCY:
- Mortality and signs of irritation: daily
- Body weight: weekly
- Haematology: monthly during first 10 months
- Urinanalysis: weekly
- Macroscopic and microscopic examination

Reliability : (2) valid with restrictions
Accepted study design. Restriction: Insufficient documentation on histopathology.

Flag : Critical study for SIDS endpoint

(27)

Type : Chronic
Species : rabbit
Sex : male/female
Strain : New Zealand white
Route of admin. : inhalation
Exposure period : 18 months
Frequency of treatm. : 6 hours/day; 5 days/week
Post exposure period : none
Doses : 1436 mg/m3 (250 ppm)
Control group : yes, concurrent vehicle
Method : other: see Reference
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Remark : The report suggests that two identical groups of males and two groups of females were exposed in addition to the unexposed control groups, corresponding to a total of six groups. As the presentation of results does not refer to absolute numbers of animals but only to percentages of total animals, their misinterpretation can be excluded.

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: no mortalities
- Signs of irritation: slight irritation on conjunctiva, no opacity of cornea, by end of third week bloody exsudate from nose indicating irritation of mucosa
- Body weight gain: no difference between exposed and control
- Haematology: no differences between exposed and control
- Urinalysis: no differences between exposed and control; pH approximately 9 in all groups
- Gross pathology: More or less pronounced hemorrhages of the lungs were observed in exposed and control animals likewise. Discoloration of the livers was also observed independent on exposure. The other organs inspected appeared normal.
- Histopathology: Lesions in lungs were found in both exposed and control animals. Microvacuolisation in livers was more pronounced in exposed animals than in controls. The other organs were normal, or changes were considered to be insignificant.

Test condition : TEST ORGANISMS
- Weight at study initiation: 2300 g
- Number of animals:
2 males and 2 females each in exposed and control groups
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 18 months
- Type of exposure: inhalation
- Vehicle: air

CLINICAL OBSERVATIONS AND FREQUENCY:
- Mortality and signs of irritation: daily
- Body weight: weekly
- Haematology: monthly during first 10 months
- Urinalysis: weekly

Reliability : (2) valid with restrictions
Accepted study design. Restriction: Insufficient documentation on histopathology.

Flag : Critical study for SIDS endpoint

(27)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : dermal
Exposure period : 8 weeks
Frequency of treatm. : daily on shaved skin
Post exposure period : yes, duration not reported
Doses : 0.1 ml; 0.2 ml
Control group : yes, concurrent no treatment
Method : other: see Reference
Year : 1976
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death:
  females: no mortalities
  males: 1/5 in all groups including control
- Body weight gain:
  males: similar in treated and untreated animals
  females: reduced by approximately 8 % in treated animals as compared to controls
- Gross pathology: formation of erythema and crust on skin after 5-6 weeks of treatment was completely reversed after end of treatment
- Histopathology: confirmation of complete disappearance of erythema and crust; no differences between exposed and untreated animals were observed in any organ

Test condition : TEST ORGANISMS
- Weight at study initiation: approximately 140 g
- Number of animals: 5 per sex and dose group incl. controls
ADMINISTRATION / EXPOSURE
- Vehicle: none
- Total volume applied: 0.1 ml (dose group 1), 0.2 ml (dose group 2)
- Doses: not precisely reported;
  0.1 ml x 921.5 mg/ml / 0.14 kg b.w. = 658 mg/kg b.w.
  0.2 ml x 921.5 mg/ml / 0.14 kg b.w. = 1316 mg/kg b.w.
- Occlusion: not reported
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: not reported
- Mortality: yes
- Body weight: yes
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: skin; others not listed
- Microscopic: skin; others not listed

Reliability : (4) not assignable
Documentation insufficient for assessment

(27)

Type	:	Sub-acute
Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	i.p.
Exposure period	:	2 w
Frequency of treatm.	:	5 injections/w
Post exposure period	:	24 h
Doses	:	230 mg/kg bw/injection
Control group	:	yes, concurrent vehicle
NOAEL	:	>= 230 mg/kg
Method	:	other: Repeated Dose Toxicity
Year	:	1989
GLP	:	no data
Test substance	:	other TS: analytical grade; dispersed in olive oil or water
Remark	:	Urinary excretion of albumin or N-acetyl-beta-glucosaminidase was not affected. Dose was equal to 10 % of the oral LD50.
Reliability	:	(4) not assignable Documentation insufficient for assessment

(6)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1535, TA1537
Test concentration	:	33, 100, 333, 1000, 3333 and 10000 (TA 1535), 100, 333, 1000, 3333, 10000 (TA 100), 33, 100, 333, 1000, 3333 (TA 98 and 1537) µg/plate
Cycotoxic concentr.	:	-S9: TA 100, 1535, 98 >=1000, TA 1537 >= 3333 µg/plate, +S9 (rat and hamster): only TA 1535 = 10000 µg/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1983
GLP	:	yes
Test substance	:	other TS: Origin: Leidy Chemical Corporation 97 % pure, 0.3 % water
Method	:	Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983): Salmonella mutagenicity test results for 250 chemicals, Environ Mutagen. 5 (Suppl. 1), 3-142
Test condition	:	SYSTEM OF TESTING - Species/cell type: from Dr. B. Ames, Univ. of California - Metabolic activation system: Aroclor 1254-induced rat and hamster liver fractions ADMINISTRATION: - Number of replicates: 3 per dose level, repeated - Positive and negative control groups and treatment: sodium azide positive for TA 1535 and TA 100 4-nitro-o-phenylenediamine positive for TA TA 98 9-aminoacridine positive for TA 97 and TA 1537 2-aminoanthracene positive all strains potassium chloride negative - Solvent: H2O 3 investigations were performed: 1. without MA 2. with MA (Arochlor-1254 liver rats)

3. with MA (Arochlor-1254 liver hamster)
Cells and test compound or solvent (water) were incubated for 20 minutes at 37 degree C in the presence of either S9 or buffer. After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37 degree C for 48 hours (Haworth et al. 1983). The analysis was performed twice, each in triplicate.

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

(97) (98)

Type : Ames test
System of testing : Salmonella typhimurium TA 1535, TA 1537, TA 1538
Test concentration : 1, 10, 100, 1000 ug/plate
Cycotoxic concentr. : Bacteriostatic tests performed on 1000, 10000 and 100000 µg/plate
Metabolic activation : with and without
Result : negative
Method : other: Huntingdon Research Centre Protocol MCB/101
Year : 1978
GLP : no data
Test substance : other TS: Elf Atochem S.A.

Test condition : Solvent: DMSO
Metabolic Activation System: Aroclor-1254 induced rat liver microsomal fraction.
Positive control:
ß-Naphthylamine (TA 1535) - 10 µg/plate
Neutral red (TA 1537) - 10 µg/plate
2-Acetylaminofluorene (TA 1538) - 20 µg/plate

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(56)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration : 10-5000 ug/plate
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other: according to Ames et al. (1975), Mutat. Res. 31, 347-364
Year : 1988
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test condition : SYSTEM OF TESTING
- Metabolic activation system:
arochlor induced rat liver S9 mix; enzymatic activity tested with aminoanthracene
ADMINISTRATION:
- Number of replicates: 2
- Application: solvent dimethyl sulfoxide
- Positive and negative control groups and treatment:
2.5 ug nitrofluorene/plate: TA 98, TA 1538 (pos.)
2.5 ug sodium azide/plate: TA 100, TA 1535 (pos.)
50 ug aminoacridine/plate: TA 1537 (pos.)
negative control: no
- Pre-incubation: yes

Test substance : Huels AG, Sample 530/880202

Reliability	:	(2) valid with restrictions Comparable to guideline study with acceptable restrictions
Flag	:	Critical study for SIDS endpoint

(61)

Type	:	Bacillus subtilis recombination assay
System of testing	:	Bacillus subtilis H17 (arg-, trp-, recE+) and M45 (arg-, trp-, recE-)
Test concentration	:	2.18 - 5.93 ug/l
Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: Liquid Cultivation-Recombination Assay
Year	:	1984
GLP	:	no data
Test substance	:	no data
Result	:	-S9: DNA damaging potential (1,38) +S9: reverse mutation (0,68)
Test condition	:	Test system: DNA repair deficient Bacillus subtilis/microsome system - H17 - recombination proficient strain - M45 - recombination defective strain Metabolic activation: Aroclor-1254 induced rat liver microsomes Evaluation criteria: R50 = CR50Rec+/CR50Rec- > 1 (DNA-damage) R50 = CR50Rec+/CR50Rec- < 1 (reverse effect) R50 =ratio of 50 % survival concentrations CR50Rec+ = concentration of the test substance giving 50% survival turbidity of Rec+ CR50Rec- = analogous
Reliability	:	(2) valid with restrictions Study well documented, acceptable for assessment. Test principle is of little relevance.

(83)

Type	:	Bacterial reverse mutation assay
System of testing	:	Photobacterium phosphoreum
Test concentration	:	no data
Cycotoxic concentr.	:	no data
Metabolic activation	:	
Result	:	negative
Method	:	other: Bioluminescence Test
Year	:	1980
GLP	:	no data
Test substance	:	no data
Test condition	:	SYSTEM OF TESTING - Deficiences/Proficiences: presumably luminescence operon - Metabolic activation system: rat liver microsomes induced by a poly-chlorinated diphenyl preparation (Chlophen-A50) - There were no information given, whether the assay was performed with or without metabolic activation.
Reliability	:	(4) not assignable Documentation insufficient for assessment.

(32)

Type	:	DNA damage and repair assay
System of testing	:	Salmonella typhimurium TA 1535/pSK 1002
Test concentration	:	680.3 ug/ml per unit of bacterial density (OD600)

Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	ambiguous
Method	:	other: umu-Test
Year	:	1985
GLP	:	no data
Test substance	:	no data
Result	:	GENOTOXIC EFFECTS: - With metabolic activation: negative (0,46) - Without metabolic activation: weakly positive (0,55)
Test condition	:	SYSTEM OF TESTING The plasmid (pSK1002) is carrying a fused gene umuC´-´lacZ: umu operon is induced by DNA-damaging agents. - Metabolic activation system: S9 fractions from male rats, pretreated with phenobarbital and 5,6-benzoflavone Evaluating system: intensity of DNA repair measured by ß-galactosidase activity (A); blank value (B) (A-B)/B > 2 ++ (strongly positive) (A-B)/B > 1 + (positive) 1 > (A-B)/B > 0,5 +- (weakly positive) 0,5 > (A-B)/B - (negative)
Reliability	:	(2) valid with restrictions Study well documented; test principle is of little relevance (101)
Type	:	Mouse lymphoma assay
System of testing	:	L5178Y/TK +/-
Test concentration	:	119.6 - 1196 µg/ml (-S9); 81.9 - 818.8 µg/ml (+S9)
Cycotoxic concentr.	:	-S9: 1656 and 2208 µg/ml; +S9: 1104 and 1472 µg/ml produced 100 % lethality
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	other TS: Exxon Corp., > 97 % pure, diluted in DMSO
Remark	:	Concentration values are calculated: Original data are given in µl/ml; a density factor of 0.92 g/cm3 is assumed.
Test condition	:	SYSTEM OF TESTING - Metabolic activation system: Aroclor 1242 and 1254 (2:1 mixture) induced liver S9 mix of male Sprague-Dawley rats ADMINISTRATION: - Dosing: 16 dose levels decreasing approximately 100fold from 100 % toxic to non-toxic; reported levels: 0.13; 0.18; 0.24; 0.32; 0.42, 0.56; 0.75; 1.0; 1.3 ul/ml (non-activated) and 0.067; 0.089; 0.12; 0.16; 0.21; 0.28; 0.38; 0.50; 0.67; 0.89 ul/ml (activated) - Number of replicates: 1 culture (controls: 2); 3 counts - Positive and negative control groups and treatment: ethyl methanesulfonate (1.0 or 0.5 ul/ml: positive) 7,12-dimethylbenz[a]anthracene (7.5 or 5.0 ug/ml: positive) DMSO only (negative) CRITERIA FOR EVALUATING RESULTS: positive: positive dose response and >= 1 of 3 highest doses had a mutant frequency 2-fold greater than background equivocal: no dose response but any dose had a mutant frequency 2-fold greater than background

Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(92) (99)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y TK+/- cells
Test concentration	:	50 - 1600 ug/ml (details see Test Conditions)
Cycotoxic concentr.	:	1600 µg/ml
Metabolic activation	:	without
Result	:	positive
Method	:	other: according to Clive and Spector, Mutat. Res. 44, 269-278 (1975) and Clive et al., Mutat. Res. 59, 61-108 (1979)
Year	:	1979
GLP	:	yes
Test substance	:	other TS: Origin: Leidy Chemical Corporation 97 % pure, 0.3 % water
Remark	:	Tennant et al (1997) judged isophorone as positive at concentrations >= 400 µg/ml without metabolic activation.
Result	:	GENOTOXIC EFFECTS: - Without metabolic activation: mutagenic Isophorone was toxic to the cultures only at moderately high concentrations. Significant increases in mutant fraction occurred in all three experiments, accompanied by a reduction of relative total growth. The LOED (lowest effective dose) was 400 ug/ml in the first experiment, where there was apparently no reduction in RTG (relative total growth) from the vehicle control level. In the second experiment, only at 600 ug/ml there was evidence of toxicity. The LOED in this experiment was 800 ug/ml. However, the cloning efficiency was low in this experiment (results questionable). In the third experiment the LOED was 800 ug/ml. CYTOTOXICITY: Relative total growth reduced at 800, 1000 and 1200 ug/ml
Test condition	:	Test procedure: Experiments were performed twice, and all doses were tested in duplicate, except the solvent control (DMSO), which was tested in quintuplicate. Cells (6 x 10e+5/ml) were treated for 4 hours at 37 degree C in medium, washed, resuspended in medium, and incubated for 48 hours at 37 degree C. After expression, 3 x10e+6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells. Trial 1: 0/50/100/200/400/800/1600 ug/ml Trial 2: 0/400/600/800/1000/1200 ug/ml Trial 3: 0/200/400/600/800/1000 ug/ml Positive control: Methylmethansulfonate
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(87) (98) (129)

Type	:	Mouse lymphoma assay
System of testing	:	Thymidine kinase (TK) locus of L5178Y tk+/- mouse lymphoma cells
Test concentration	:	no data
Cycotoxic concentr.	:	up to concentration: 10-20% RS

Metabolic activation	:	with and without
Result	:	ambiguous
Method	:	other: Microwell method
Year	:	1999
GLP	:	no data
Test substance	:	no data
Remark	:	Mouse lymphoma assay in the short treatment (3 hr) was not as sensitive as the chromosomal aberration assay. Validation study - 45 japanese and 7 overseas laboratories are involved.
Result	:	- With metabolic activation: inconclusive (positive in one lab, dose-dependent positive response * (max. Mutat. frequency < 2 times the spontanous one)) - Without metabolic activation: negative * statistically significant
Test condition	:	Isophorone was tested in two labs. Test: 3 test concentrations (2 replicates); 3-6 hr-treatment Solvent: DMSO Metabolic activation: rat livers after phenobarbital- and 5,6-benzoflavone treatment Positive control (-S9): methylmethansulfonate (10 µg/ml) Positive control (+S9): cyclophosphamide (3 µl/ml)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(53)

Type	:	Mouse lymphoma assay
System of testing	:	Thymidine kinase (TK) locus of L5178Y tk+/- mouse lymphoma cells
Test concentration	:	0-1500 µg/ml (taken out of graph)
Cycotoxic concentr.	:	1500 µg/ml (taken out of graph)
Metabolic activation	:	without
Result	:	positive
Method	:	other: Microwell method with long-term treatment
Year	:	1999
GLP	:	no data
Test substance	:	no data
Remark	:	Longer treatment of cells (24 h) may detect some clastogens and spindle poisons, who are not detected after short term treatments. Validation study
Result	:	Estimation out of graphic: Positive mutant frequency is observed at concentrations of >= 1300 µg/ml.
Test condition	:	Treatment of cells: 24 h Solvent: DMSO Positive control (-S9): methylmethansulfonate (10 µg/ml) Positive control (+S9): cyclophosphamide (3 µl/ml)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(54)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y TK+/- mouse lymphoma cell clone 3.7.2C
Test concentration	:	ca. 0 - 1600 ug/ml
Cycotoxic concentr.	:	tested up to concentration: 10-20% RS (relative survival)
Metabolic activation	:	with and without

Result	:	ambiguous
Method	:	other: microwell method
Year	:	1996
GLP	:	no data
Test substance	:	no data
Result	:	GENOTOXIC EFFECTS: - With metabolic activation: inconclusive (positive in one lab) - Without metabolic activation: negative
Test condition	:	Isophorone was tested in two laboratories SYSTEM OF TESTING - Metabolic activation system: S9 mix, no details reported ADMINISTRATION: - Number of replicates: 2 (laboratories) - Positive and negative control groups and treatment: not reported
Reliability	:	(4) not assignable Documentation insufficient.

(121)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y TK+/- mouse lymphoma cells, in situ variant
Test concentration	:	no data
Cycotoxic concentr.	:	no data
Metabolic activation	:	no data
Result	:	positive
Method	:	other: no details reported
Year	:	1993
GLP	:	no data
Test substance	:	no data
Result	:	The mutation frequency was at least 2.5-over 8 times higher in the in situ variant of the Mouse lymphoma assay than observed in the standard procedure reported by Tennant, R.W.; Margolin, B.H.; Shelby, M.D.; Zeiger, E.; Haseman, J.K.; Spalding, J.; Caspary, W.; Resnick, M.; Stasiewicz, S.; Anderson, B.; Minor, R. (1987), Science 236, 933-941 No details reported.
Reliability	:	(4) not assignable Abstract

(95)

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster Ovary (CHO) cells
Test concentration	:	- S9: 50-1600 µg/ml, + S9: 250-1500 µg/ml
Cycotoxic concentr.	:	>= 5000 µg/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: see Method freetext
Year	:	1989
GLP	:	yes
Test substance	:	other TS: origin: Leidy, purity: 97.2 %
Method	:	Galloway, S.M. et al., Environ Mol. Mutagen. 10 (10), 1-175, with a few modifications, see reference for details
Remark	:	Statement of GLP compliance is given in Appendix N of the NTP report.
Test condition	:	Solvent: serum-free culture medium S-9 mix: Aroclor 1254-induced male Sprague Dawley rats

Analysis: 100 or 200 cells were scored for each dose (cells with chromosome number lower than 19 or higher than 23 were excluded)
Test procedure:
In the absence of S9, CHO cells were incubated with test compound or solvent for 8-10 hours at 37 degree C. Cells were then washed, and fresh medium containing colcemid (0.1 ug/ml) was added. After a further 2-3 hours of incubation, cells were harvested by mitotic shake-off, fixed, and stained in 6 % Giemsa.
In the presence of S9, cells were incubated with test compound or solvent for 2 hours at 37 degree C. Cells were then washed, medium was added, and incubation continued for 8-10 hours. Colcemid (0.1 ug/ml) was added for the last 2-3 hours of incubation; then cells were harvested and fixed as above.
2 independent experiments:
-S9 mix: 50, 160, 500, 1600 ug/ml
-S9 mix: 250, 500, 1000, 1600 ug/ml
+S9 mix: 250, 500, 1000 ug/ml
+S9 mix: 750, 1000, 1250, 1500 ug/ml

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

(43) (98) (129)

Type : Chromosomal aberration test
System of testing : Chinese Hamster lung fibroblast cells (CHL/IU)
Test concentration : standard procedure: 0.25-1.25 mg/ml; modified treatment: 0.75 - 1.75 mg/ml
Cycotoxic concentr. : standard procedure: -S9 (24 h): 1.25 mg/ml, -S9 (48 h): 1.0 mg/ml; modified treatment: -S9: > 1.25 mg/ml (not given), +S9: 1.75 mg/ml
Metabolic activation : with and without
Result : ambiguous
Method : other
Year : 1996
GLP : no data
Test substance : other TS: Source: Tokyo Kasei Purity: > 98 %

Result : Standard treatment:
Negative after 24 and 48 hours treatment

Modified treatment:
without S9: positive at 1.25 mg/ml (highest concentration tested)
with S9: positive at 1.5 mg/ml

Test condition : SYSTEM OF TESTING
- Metabolic activation system: Phenobarbital and 5,6-Benzoflavone pretreated liver S9-fraction
- No. of metaphases analyzed: 100/dose

ADMINISTRATION:
- Positive and negative control groups and treatment:
  negative: DMSO
  positive: mitomycin C, cyclophosphamide

Standard procedure:
- without metabolic activation (24 h)
- without metabolic activation (48 h)

Modified treatment: Cells are treated for 6 h and then

cultured in fresh medium for another 18 h.
- without metabolic activation
- with metabolic activation

CRITERIA FOR EVALUATING RESULTS:
positive: >= 10 % polyploidy or structural aberrations
marginal: 5-10 %
negative: < 5 %

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(84)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese Hamster Ovary (CHO) cells
Test concentration	:	5 - 1600 µg/ml
Cycotoxic concentr.	:	>= 500 µg/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: according to NTP protocol
Year	:	1986
GLP	:	yes
Test substance	:	other TS: Origin: Leidy Chemical Corporation 97 % pure, 0.3 % water

Result : Isophorone induced increased SCE frequencies only without metabolic activation at cytotoxic concentrations >= 500 µg/ml.

Test condition : In the absence of S9, CHO cells were incubated with test compound or solvent for 2 hours at 37 degree C. Then BrdU was added, and incubation continued for 27-35 hours. Cells were washed, fresh medium containing BrdU (10 uM) and colcemid (0.1 ug/ml) was added, and incubation was continued for 2-3 hours. Cells were then collected by mitotic shake-off, treated for 3 minutes with potassium chloride (75 mM), washed twice with fixative, and dropped onto slides and air-dried. Staining was by a modified technique (after Perry and Wolff 1974; Goto et al. 1978)

In the presence of S9, cells were incubated with test compound or solvent for 2 hours at 37 degree C. Then cells were washed, and medium containing 10 uM BrdU was added. Cells were incubated for a further 26 hours, with colcemid (0.1 ug/ml) present for the final 2-3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(43) (98) (129)

Type	:	other: Transformation assay
System of testing	:	A31-1-13 clone of BALB/c-3T3 cells
Test concentration	:	46-738 mg/l
Cycotoxic concentr.	:	716 mg/l (50 % reduction of relative cloning efficiency)
Metabolic activation	:	without
Result	:	positive
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	other TS: Origin: Radian Corporation, Austin, TX 78766, USA

Remark	:	No endpoint of genotoxicity
Test condition	:	SYSTEM OF TESTING - Species/cell type: A31-1-13 clone of BALB/c-3T3 cells ADMINISTRATION: - Positive and negative control groups and treatment: positive: benzo(a)pyrene negative: untreated CRITERIA FOR EVALUATING RESULTS: (1) piling and overlapping cells (2) disorientation of cells at the periphery of the focus (3) invasion of transformed cells into a contact-inhibited monolayer of wild type cells criteria (1) through (3) have to be fulfilled. (4) Evaluation of the transforming activity on the basis of statistical significance (see reference)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(85)

Type	:	Unscheduled DNA synthesis
System of testing	:	primary liver cell cultures from adult male Sprague-Dawley rats
Test concentration	:	0.005 - 0.4 µl/ml (corresponds to 4.6 - 368 µg/ml)
Cycotoxic concentr.	:	100 % toxicity: 0,4 µl/ml
Metabolic activation	:	without
Result	:	negative
Method	:	other: based on Williams, G.M. (1976), Cancer Lett. 1, 231-236
Year	:	1984
GLP	:	yes
Test substance	:	other TS: Exxon Corp., > 97 % pure, diluted in ethanol
Result	:	GENOTOXIC EFFECTS: no significant increase CYTOTOXIC CONCENTRATION: - Without metabolic activation: In the initial cytotoxicity test, relative toxicity (RT) of 95.95 % at 0.5 ml/l and 4.05 % at 0.005 ml/l was observed.
Test condition	:	ADMINISTRATION: - Dosing: - Dosing: 0.005; 0.01; 0.05; 0.10; 0.20; 0.40 ul/ml - Number of replicates: 3 - Application: dissolved in ethanol - Positive and negative control groups and treatment: positive: 2-acetyl aminofluorene (2 and 20 µl/ml) negative: ethanol CRITERIA FOR EVALUATING RESULTS: - positive: dose-related response and significant increase in average net nuclear grains or significant increase in average net nuclear grains for >=2 successive doses - significant increase: >= 5fold over solvent control - marginal positive: significant increase in average net nuclear grains for 1 dose Dose-related response plus at least one dose with significant increase = Test article judged positive.
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(93) (99)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Drosophila SLRL test
Species	:	Drosophila melanogaster
Sex	:	male
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	72 hours
Doses	:	2,000 ppm
Result	:	negative
Method	:	other: NTP SLRL test method
Year	:	1983
GLP	:	no data
Test substance	:	other TS: Origin: Leidy; lot/batch 1204/01 analytical purity: 97.2 %
Result	:	MORTALITY: - feeding: 15 % - injection: 47 % PERCENT STERILITY: - feeding, injection: 0 % PERCENT LETHALS: - feeding: 0.11 %; control: 0.18 % - injection: 0.22 %; control: 0.17 % RESULT: - feeding, injection: negative
Test condition	:	TEST ORGANISMS: - Age: adult - Origin: Canton S and Basc stocks maintained at Brown University and the University of Wisconsin ADMINISTRATION: - Vehicle: ethanol, CAS RN 64-17-5 / 5 % sucrose solution - Duration of test: first mating after 72 hours of exposure - Frequency of treatment: - Sampling times and number of samples: three broods, for each >= ca. 5000 chromosomes scored unless mutant frequency > 1.0 % - Control groups and treatment: concurrent solvent EXAMINATIONS: - Criteria for evaluating results: mutation frequency > 0.15 % (P < 0.05): positive mutation frequency > 0.10 % (P < 0.01): positive mutation frequency 0.10-0.15 % (P 0.01-0.05): equivocal other: negative FOLLOW-UP TESTING: 2-3-day-old males were injected with 0.7 % NaCl solution containing the test chemical at 12,500 ppm. At 24 hours postinjection, toxicity was tested and survivors were mated.
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(38)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	i.p.

Exposure period	:	12, 24, 48 hours
Doses	:	496.8 mg/kg (= M.T.D./LD20) in corn oil as 10 ml solution/kg bw
Result	:	negative
Method	:	other: Micronucleus Cytogenetic Assay
Year	:	1988
GLP	:	yes
Test substance	:	other TS: Exxon Corp., > 97 % pure, diluted in corn oil
Test condition	:	TEST ORGANISMS: - Age: 6-8 weeeks, Charles River Labs., Kingston, NY - No. of animals per dose: 5 males / females per dose and test duration ADMINISTRATION: - Vehicle: corn oil - Duration of test: 12; 24; 48 hours - Frequency of treatment: single application - Sampling times and number of samples: 12, 24, 48 h post dosing - Control groups and treatment: positive: 0.25 mg/kg triethylene melamine, 24 hours solvent: corn oil EXAMINATIONS: - Organs examined: femur bone marrow - Criteria for evaluating results: 1-way analysis of variance and Duncan's multiple range test ($P <= 0.05$) - Criteria for selection of M.T.D.: LD20
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail.
Flag	:	Critical study for SIDS endpoint

(91) (99)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	other: CFLP
Route of admin.	:	gavage
Exposure period	:	2 equal exposures (half of total dose) separated by interval 24 hours
Doses	:	450; 900; 1800 mg/kg bw respectively (total)
Result	:	negative
Method	:	other: no data
Year	:	1978
GLP	:	no data
Test substance	:	other TS: Elf Atochem S.A.
Result	:	MORTALITY: 2 females and 1 male within 6 h after second dose in 1800 mg/kg bw group CLINICAL SIGNS: 1800 mg/kg bw: period of lethargy MICRONUCLEATED CELL COUNT: neg. control: mean 2.0; range 0-5 pos. control: mean 82.5; range 68-107 450 mg/kg bw: mean 2.2; range 0-5 900 mg/kg bw: mean 1.9; range 0-6 1800 mg/kg bw: mean 2.1; range 1-4 Six hours after the last treatment, the mean micronucleated cell counts and the bone marrow cytotoxicity were similar in all test groups and controls.
Test condition	:	TEST ORGANISMS: - Source: Anglia Laboratory Animals, Alconbury, Huntingdon

- Weight at study initiation: 19-23 g
- No. of animals per dose: 5 male, 5 female
ADMINISTRATION:
- Vehicle: 1 % methylcellulose
- Duration of test: mice killed 6 hours after second dose
- Sampling times and number of samples: 6 h after dose # 2
- Control groups and treatment:
negative: vehicle
positive: 14 mg/kg bw Mitomycin C (i.p. injection)
EXAMINATIONS:
- Clinical observations: daily
- polychromatic erythrocytes counted per mouse: 2000

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(55)

5.7 CARCINOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	103 weeks
Frequency of treatm.	:	5 days/week
Post exposure period	:	none
Doses	:	250 and 500 mg/kg bw d
Result	:	ambiguous
Control group	:	yes, concurrent vehicle
Method	:	other: NTP-Carcinogenicity Study
Year	:	1986
GLP	:	no
Test substance	:	other TS: Origin: Leidy Chemical Corporation Lot A (months 1-6): 97 % pure, 0.3 % water Lot B (rest) 94 % pure, 1.4 % water
Remark	:	The kidney tumors are probably attributed to an a2u-globulin mechanism, see additional remarks (chapter 5.11).
Result	:	MORTALITY AND TIME TO DEATH: The overall survival rate was low: Males: 33 (control) / 33 (250 mg/kg) / 14 (500 mg/kg) Females: 30 (control) / 23 (250 mg/kg) / 20 (500 mg/kg) Non-accidental deaths: Males: 13 (control) / 12 (250 mg/kg) / 30 (500 mg/kg) Females: 19 (control) / 21 (250 mg/kg) / 16 (500 mg/kg) 500 mg/kg bw, m: increased mortality (after week 98) Deaths related to gavage error increased with dose in females. CLINICAL SIGNS: No compound-related clinical signs were observed. BODY WEIGHT GAIN: 500 mg/kg bw, m: body weight was 5% lower (after week 1) 500 mg/kg bw, f: body weight was 8% lower (after week 43) HISTOPATHOLOGY: Kidneys; m (controls, 250, 500 mg/kg bw): - tubular cell hyperplasia (0/50, 1/50*, 4/50*)

- tubular cell adenoma: 0/50, 0/50, 2/50
- tubular cell adenocarcinoma: 0/50, 3/50, 1/50
- epithelial hyperplasia of the renal pelvis (0/50, 5/50*, 5/50*)
- tubule mineralization (1/50, 31/50, 20/50)
- Nephropathy (49/50, 47/50, 46/50) - with higher severity in low dose males

Kidneys, f:
- Nephropathy (21/50, 39/50*, 32/50*)
No further compound-related findings in kidneys of females.

Adrenal cortex, m:
- fatty metamorphosis (7/50, 21/50, 26/50) (lesions in which adrenal cortical cells contained cytophalsmic vacuoles)

Preputial gland, m:
- carcinoma (0/50, 0/50, 5/50*) - Lesions were noted macroscopically and generally were greater than 1 cm. Histopathology of preputial gland was only performed on high dose group.

Clitoral gland, f:
- adenomas (0/50, 2/50, 0/50) - These lesions were histogenically related to the preputial gland carcinomas observed in male rats, providing some support for an association of isophorone exposure with this tumor type.

Anterior Pituitary, f:
- focal hyperplasia (3/49, 6/48*, 13/47*)
But incidence of adenomas occurred with a negative trend in female rats (21/49, 17/48, 12/47)

* statistically significant

Conclusion:
Some evidence of carcinogenicity is observed in male rats (renal tubular cell adenomas and adenocarcinomas, carcinomas of the preputial gland).
No evidence of carcinogenicity is observed in female rats.

Test condition **:** TEST ORGANISMS
- Age: 6-7 weeks
- Number of animals: 50 per dose group and sex
ADMINISTRATION / EXPOSURE
- Vehicle: corn oil
- Concentration in vehicle: 50 and 100 mg/ml, respectively
- Total volume applied: 5 ml/kg
CLINICAL OBSERVATIONS AND FREQUENCY
- Body weight: weekly for first 13 weeks, then monthly
- Clinical signs: weekly for first 13 weeks, then monthly
- Mortality: twice daily
- Macroscopic examination: twice daily
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: no details reported
- Microscopic:
gross lesions and tissue masses, skin, mammary gland, thymus, heart, lungs and bronchi, trachea, thyroid gland, parathyroids, esophagus, stomach, colon, small intestines, mesenteric lymph node, pancreas, spleen, liver, kidneys, adrenal glands, urinary bladder, prostate/testes or ovaries/uterus, brain, pituitary gland, eyes (if grossly abnormal), thoracic vertebrae, including bone marrow and

spinal cord
STATISTICAL METHODS:
- mortalities: life table analysis
- tumor incidence: life table analysis, incidental tumor test

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

(12) (98)

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	gavage
Exposure period	:	103 weeks
Frequency of treatm.	:	5 days/week
Post exposure period	:	none
Doses	:	250 and 500 mg/kg bw d
Result	:	ambiguous
Control group	:	yes, concurrent vehicle
Method	:	other: NTP-Carcinogenicity Study
Year	:	1986
GLP	:	no
Test substance	:	other TS: Origin: Leidy Chemical Corporation Lot A (months 1-6): 97 % pure, 0.3 % water Lot B (rest) 94 % pure, 1.4 % water

Result :

MORTALITY AND TIME TO DEATH:
The overall survival rate was:
Males: 13 (control) / 13 (250 mg/kg) / 18 (500 mg/kg)
Females: 24 (control) / 33 (250 mg/kg) / 34 (500 mg/kg)
Non-accidental deaths:
Males: 28 (control) / 34 (250 mg/kg) / 29 (500 mg/kg)
Females: 23 (control) / 14 (250 mg/kg) / 11 (500 mg/kg)
>= 250 mg/kg bw f: decrease mortality (non-accidental kills: 23/50, 14/50, 11/50).
Further deaths are documented to be due to gavage accidents.

CLINICAL SIGNS:
No compound-related clinical signs were observed.

BODY WEIGHT GAIN:
500 mg/kg bw f: body weight 5% lower (during the 2nd year)

HISTOPATHOLOGY:
Liver, m:
Hepatocellular adenoma: 6/48, 7/50, 13/50*
Hepatocellular carcinoma: 14/48, 13/50, 22/50*
Hepatocellular adenoma or carcinoma: 18/48, 18/50, 29/50*
Coagulative necrosis: 3/48, 10/50, 11/50
Hepatocytomegaly: 23/48, 39/50, 37/50

Liver, f:
Hepatocellular adenoma or carcinoma: 4/50, 6/50, 8/50

Skin, m:
Fibroma, Sarcoma, Fibrosarcoma or neurofibrosarcoma: 6/48, 8/50, 14/50 (P = 0,050)

Hematopoietic system, m:
Lymphoma or Leukemia: 8/48, 18/50*, 5/50

Lung, m:
Alveolar/bronchiolar adenoma or carcinoma: 7/47, 1/50, 3/50 (significantly negative trend)

Kidney, m:
Chronic focal inflammation: 7/48, 18/50, 21/50
Nephropathy: 16/48, 15/50, 9/50

Pituitary gland, f:
Hyperplasia: 5/47, 7/41, 13/44
Adenoma or adenocarcinoma: 16/47, 13/41, 4/44 (significant negative trend)

* P < 0,05

Conclusion: Equivocal evidence of carcinogenicity in male mice (increase in hepatocellular und integumentary tumors). No evidence of carcinogenicity is observed in female rats.

Test condition : TEST ORGANISMS
- Age: 6-8 weeks
- Number of animals: 50 per dose group and sex

ADMINISTRATION / EXPOSURE
- Vehicle: corn oil
- Concentration in vehicle: 25 and 50 mg/ml, respectively
- Total volume applied: 10 ml/kg

CLINICAL OBSERVATIONS AND FREQUENCY
- Body weight: weekly for first 13 weeks, then monthly
- Clinical signs: weekly for first 13 weeks, then monthly
- Mortality: twice daily
- Macroscopic examination: twice daily

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: no details reported
- Microscopic:
gross lesions and tissue masses, skin, mammary gland, thymus, heart, lungs and bronchi, trachea, thyroid gland, parathyroids, esophagus, stomach, colon, small intestines, mesenteric lymph node, pancreas, spleen, liver, gallblader, kidneys, adrenal glands, urinary bladder, prostate/testes or ovaries/uterus, brain, pituitary gland, eyes (if grossly abnormal), thoracic vertebrae, including bone marrow and spinal cord

STATISTICAL METHODS:
- mortalities: life table analysis
- tumor incidence: life table analysis, incidental tumor test

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

(12) (98)

5.8.1 TOXICITY TO FERTILITY

Type : One generation study
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : inhalation
Exposure period : 6 hours/day

Frequency of treatm.	:	5 days/week
Premating exposure period		
Male	:	3 months
Female	:	3 months
Duration of test	:	females: 4 months; males: 6 months
No. of generation studies	:	
Doses	:	500 ppm = 2873 mg/m3 (saturation)
Control group	:	yes, concurrent vehicle
Method	:	other: See Reference
Year	:	1976
GLP	:	no data
Test substance	:	other TS: Elf Atochem S.A.
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Parental data and F1: - Body weight: no difference between exposed and control - Description, severity, time of onset and duration of clinical signs: irritation of eyes and nose (exposed) - Mortality: no mortalities in control groups 1/10 of exposed females and 2/10 of exposed males died - Gross pathology incidence and severity: traces of bleeding in lungs of both exposed and control animals - Histopathology incidence and severity: slight to medium congestion in lungs with similar intensity in exposed and control; granular state and clarification of liver cytoplasma with similar intensity in exposed and control - Offspring toxicity F1 and F2: - Litter size and weights: 7-10 per female, normal behaviour, none dead - Post natal survival until weaning: no difference between exposed and control - Effects on offspring: no difference between exposed and control observed at necropsy
Test condition	:	TEST ORGANISMS - Weight at study initiation: approximately 140 g - Number of animals: 10 males, 10 females ADMINISTRATION / EXPOSURE - Type of exposure: inhalation - Vehicle: air - Control group: 10 males, 10 females MATING PROCEDURES: - after three months of exposure overnight mating of 5 exposed males with 5 exposed females 5 exposed males with 5 control females 5 control males with 5 exposed females 5 control males with 5 control females - next morning exposure continued for exposed animals (females until littering) PARAMETERS ASSESSED DURING STUDY P AND F1: - Clinical observations: behaviour, body weight development, mortality of P, number and vitality of F1 Limitations of the study: Only one dose tested Small group size No information on mating success
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific

principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.07.2003 (27)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	6th to 15th day of gestation
Frequency of treatm.	:	6 h/d
Duration of test	:	section on the 20th d of gestation
Doses	:	144, 289 and 664 mg/m3 (corresponds to 25, 50 and 115 ppm)
Control group	:	other: yes, concurrent conditioned air
NOAEL maternal tox.	:	= 289 mg/m³
NOAEL teratogen.	:	>= 664 mg/m³
Method	:	other: Teratogenicity Test
Year	:	1984
GLP	:	no data
Test substance	:	other TS: purity 96.8 %

Result :

NOAEL (maternal): based on reduced body weight, clinical signs are not considered

MATERNAL TOXIC EFFECTS BY DOSE LEVEL:
- Mortality and day of death: no mortalities
- Number pregnant per dose level: 22
- Number of resorptions: no statistically significant differences between treated and control groups
- Number of implantations: no statistically significant differences between treated and control groups
- Number of corpora lutea: no statistically significant differences between treated and control groups
- Duration of Pregnancy: no statistically significant differences between treated and control groups
- Body weight: reduced in days 12 (-6.1 %) and 15 (-6.8 %) rats in 664 mg/m3 dose group
- Food/water consumption: reduced food consumption in 664 mg/m3 dose group
- Clinical signs: alopecia and cervical or anogenital staining (each dose-related).

FETAL DATA:
No statistically significant differences between treated and control groups:
- Litter size and weights
- Number viable
- Sex ratio
- Grossly visible abnormalities
- External abnormalities
- Soft tissue abnormalities
- Skeletal abnormalities

Test condition :

TEST ORGANISMS
ADMINISTRATION / EXPOSURE
- Vehicle: no vehicle
- Concentrations: 0 / 25 / 50 / 115 ppm
- Type or preparation of particles: vapor

MATING PROCEDURES: Virgin female Fischer rats (approximately

11 weeks of age) were paired with similar males for mating. Females were confirmed to have mated by observation of a copulatory plug in the vagina or by observation of sperm in a vaginal rinse.
PARAMETERS ASSESSED DURING STUDY:
- Body weight gain: each 3rd day
- Food consumption: 3 day intervals
- Clinical observations: each 3rd day
- Examination of uterine content: identified as live fetuses, dead fetuses, late resorptions, and early resorptions at end of study (day 20 of gestation). The uterus of each animal was stained in 10 % aqueous ammonium sulfide and further examined for confirmation of implantation sites. Corpora lutea were counted.
- Examination of fetuses: Live and dead fetuses were weighed, examined externally for gross abnormalities, and crown-rump distances were determined. Further examinations: Skeletal malformations and ossification variations.
STATISTICAL METHODS:
- Bartlett's test of homogeneity of variance: body weight, body weight change, food consumption, number of implantation sites, ratio of live fetuses to implantation sites, ratios of resorptions to implant sites, malformations per litter.
- Kruskal-Wallis test if variances were not equivalent.
- Standard nested analysis of variance for fetal weights.

Conclusion : The test material elicited a clinical effect in the pregnant dams in the form of decreased food consumption (115 ppm, days 6-20 and 0-20), lower body weights (115 ppm, days 12G and 15G), and dose related increases in alopecia and staining of the cervical and anogenital areas.

During the conduct of the probe study there was one instance of exencephaly noted in a rat fetus. Based on the observations made in this study the authors do not believe that this anomaly was related to the test material.

Within the framework of the dose levels and test methods used, the test material was not teratogenic or fetotoxic.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

08.07.2003 (36)

Species : mouse
Sex : female
Strain : CD-1
Route of admin. : inhalation
Exposure period : 6th to 15th day of gestation
Frequency of treatm. : 6 h/d
Duration of test : section on the 18th d of gestation
Doses : 144, 289 and 664 mg/m3 (corresponds to 25, 50 and 115 ppm)
Control group : other: yes, concurrent conditioned air
NOAEL maternal tox. : = 289 mg/m³
NOAEL teratogen. : >= 664 - mg/m³
Method : other: Teratogenicity Test
Year : 1984
GLP : no data
Test substance : other TS: purity 96.8 %

Result : NOAEL (maternal): based on reduced body weight

MATERNAL TOXIC EFFECTS BY DOSE LEVEL:
- Mortality and day of death: no mortalities
- Number pregnant per dose level: 22
- Number of resorptions: no statistically significant differences between treated and control
- Number of implantations: no statistically significant differences between treated and control
- Number of corpora lutea: no statistically significant differences between treated and control
- Duration of Pregnancy: not statistically significant differences between treated and control
- Body weight: reduced in day 18 mice in 664 mg/m3 dose group (-5.6 %, corrected for uterine weight)
- Clinical signs: Unremarkable

FETAL DATA:
- Litter size and weights: no statistically significant differences between treated and control
- Number viable: no statistically significant differences between treated and control
- Sex ratio: no statistically significant differences between treated and control
- Grossly visible abnormalities: no statistically significant differences between treated and control
- External abnormalities: no statistically significant differences between treated and control
- Soft tissue abnormalities: no statistically significant differences between treated and control
- Skeletal abnormalities (control, 144, 289, 664 mg/m3): 24/106, 26/120, 31/111, 36/110 no statistically significant differences between treated and control.

- Other examinations: Three instances of exencephaly were noted in mouse fetuses (no data on doses). These effects are regarded as not to be compound-related by the authors.

Test condition **:** TEST ORGANISMS
ADMINISTRATION / EXPOSURE
- Vehicle: no vehicle
- Concentrations: 0 / 25 / 50 / 115 ppm
- Type or preparation of particles: vapour
MATING PROCEDURES: Virgin female CD-1 mice (approximately 9 weeks of age) were paired with similar males for mating. Females were confirmed to have mated by observation of a copulatory plug in the vagina or by observation of sperm in a vaginal rinse.
PARAMETERS ASSESSED DURING STUDY:
- Body weight gain: each 3rd day
- Food consumption: not determined
- Clinical observations: each 3rd day
- Examination of uterine content: identified as live fetuses, dead fetuses, late resorptions, and early resorptions at end of study (day 18 of gestation). The uterus of each animal was stained in 10 % aqueous ammonium sulfide and further examined for confirmation of implantation sites. Corpora lutea were counted.
- Examination of fetuses: Live and dead fetuses were weighed, examined externally for gross abnormalities, and crown-rump distances were determined.
- Further examinations: Skeletal malformations and ossification variations.
STATISTICAL METHODS:

- Bartlett's test of homogeneity of variance: body weight, body weight change, number of implantation sites, ratio of live fetuses to implantation sites, ratios of resorptions to implant sites, malformations per litter.
- Kruskal-Wallis test if variances were not equivalent.
- Standard nested analysis of variance for fetal weights.

Conclusion : The test material elicited a clinical effect in the pregnant dams in the form of lower body weights (115 ppm, day 18G).

During the conduct of the probe study there were three instances of exencephaly noted in mouse fetuses. Based on the observations made in this study the authors do not believe that these anomalies were related to the test material.

Within the framework of the dose levels and test methods used, the test material was not teratogenic or fetotoxic.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

08.07.2003 (36)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type : other: Sub-chronic
In vitro/in vivo : In vivo
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : gavage
Exposure period : 13 weeks
Frequency of treatm. : 5 days/week
Duration of test : 13 weeks
Doses : 62.5, 125, 250, 500, or 1000 mg/kg bw d
Control group : yes, concurrent vehicle
Result : No compound-related changes in reproductive organs are reported.
Method : other
Year : 1986
GLP : no data
Test substance : other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water

Test condition : Details of this study are described in chapter 5.4.
Histopathological examinations in reproductive organs were performed: mammary gland, seminal vesicles, prostate/testes or ovaries/uterus

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ weight determination

Flag : Critical study for SIDS endpoint

(98)

Type : other: Sub-chronic
In vitro/in vivo : In vivo
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage

Exposure period	:	13 weeks
Frequency of treatm.	:	5 days/week
Duration of test	:	13 weeks
Doses	:	62.5, 125, 250, 500, or 1000 mg/kg bw d
Control group	:	yes, concurrent vehicle
Result	:	No compound-related changes in reproductive organs are reported.
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	other TS: Origin: Leidy Chemical Corporation, 97 % pure, 0.3 % water
Test condition	:	Details of this study are described in chapter 5.4. Histopathological examinations in reproductive organs were performed: mammary gland, seminal vesicles, prostate/testes or ovaries/uterus
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: No organ weight determination
Flag	:	Critical study for SIDS endpoint

(98)

Type	:	other: Sub-chronic
In vitro/in vivo	:	In vivo
Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	other: gelatin capsules
Exposure period	:	90 days
Frequency of treatm.	:	daily
Duration of test	:	90 days
Doses	:	35, 75 and 150 mg/kg bw d
Control group	:	yes, concurrent vehicle
Result	:	No compound-related changes in reproductive organs are reported.
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	other TS: Origin: International Chemical Corp., New York, 11 Aug 1971
Test condition	:	Details of this study are described in chapter 5.4. Histopathological examinations in reproductive organs were performed: Males: testes, prostate, seminal vesicles; female: ovary, uterus, mammary gland
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(109)

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience	:	other: human sensory irritation
Remark	:	human sensory irritation
Result	:	positive observations: odor detection: 6/6 at 100 mg/m3

nasal irritation: 4/6 at 377 mg/m3; 5/6 at 513 mg/m3
eye irritation: 1/6 at 377 mg/m3; 1/6 at 513 mg/m3
throat irritation: 1/6 at 199 mg/m3; 2/6 at 377 mg/m3
mask removal: 1/6 at 513 mg/m3; 1/6 at 595 mg/m3
second series following week: no significant difference

Conclusions:
Throat irritation < 35 ppm (199 mg/m3)
Eye and nasal irritation < 64 ppm (359 mg/m3)

Test condition : PERSONS EXPOSED: 6
EXPOSURE
- Reason of exposure:
determination of human sensory irritation thresholds
- Type of exposure: gaseous via face mask
- Duration of exposure: 7 minutes per concentration
- Exposure concentrations / dose:
week 1: 100; 199; 377; 513 mg/m3
week 2: 359; 595 mg/m3
- Criteria:
1. Detection of odor
2. Nose irritation
3. Eye irritation
4. Throat irritation (instead of skin irritation)
5. Mask removal
- Other information:
The test substance did not vaporize immediately, but as there was no deposition on the walls of the aerosol chamber, complete vaporization was assumed. Nominal concentrations were reported.

Test substance : Origin: Esso Research and Engineering Company, 21 Dec 1964, used as received

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint
27.06.2003 (34)

Type of experience : other: effects of occupational exposure

Result : Workers exposed to isophorone at levels of 5-8 ppm (29-46 mg/m3) for 1 month complained of fatigue and malaise. When levels were reduced to 1-4 ppm (6-23 mg/m3), the complaints ceased.

Reliability : (4) not assignable
Documentation insufficient for assessment
(140) (145)

Type of experience : other: tolerable level in the air

Result : 200 ppm (1150 mg/m3) for 1 minute are intolerable for humans.
40 ppm (230 mg/m3) for 4 minutes were intolerable to 50 % human volunteers.

Isophorone did not cause allergic contact sensitization (0/10 human volunteers).

Reliability : (4) not assignable
Documentation insufficient for assessment
(138) (139)

Type of experience : other: tolerable level in the air

Remark : The concentration of isophorone in the exposure chamber was calculated not analytically measured.
Result : 144 mg/m3 (25 ppm): eye, nose and throat irritation; odor objection by 70%.
58 mg/m3 (10 ppm): odor objection by 40%;

The highest tolerable level for 8 hour exposure was judged to be 58 mg/m3 (10 ppm) by a majority of the test persons.
Test condition : Ca. twelve subjects of both sexes were exposed to atmospheric isophorone for fifteen minutes. Motion pictures were shown to occupy their attention.
Test substance : Technical grade, supplied by Shell Development Company, Emeryville, California
Reliability : (2) valid with restrictions
Although insufficient in documentation delivers this investigation relevant data on human exposure to isophorone.
Flag : Critical study for SIDS endpoint

(116)

Type of experience : other: human sensory irritation

Result : 230, 490, 1,150, and 2,300 mg/m3: eye, nose, and throat irritation, narcotic action.

230, 490 mg/m3: symptoms decreased during exposure

1150, 2300 mg/m3: few complaints of nausea, headache, dissizess, faintness, inebriation, and a feeling of suffocation All symptoms decreased during exposure.
Test condition : Groups of 11 or 12 subjects were exposed for a few minutes to measured concentrations of 230, 490, 1,150, and 2,300 mg/m3, corresponds to 40, 85, 200, 400 ppm, isophorone in a small room.
Test substance : commercial grade, 10 % boiling below 212 degree C, 98 % boiling below 219 degree C.
Reliability : (2) valid with restrictions
Although insufficient in documentation delivers this investigation relevant data on clinical signs after exposure of isophorone to humans. Significant methodological deficiencies: The concentrations reported in other parts of this publication are higher than would be possible under the reported conditions.
Flag : Critical study for SIDS endpoint

(117)

Type of experience : Human

Result : Unspecified exposure to isophorone caused a corneal burn which healed rapidly.
Reliability : (4) not assignable
Documentation insufficient

(88)

5.11 ADDITIONAL REMARKS

Type : adsorption

Result : blood concentrations after
10 min: 0-102 µg/ml
30 min: 75-141 µg/ml
1 h: 88-94 µg/ml
2 h: 70-77 µg/ml
3 h: 50-56 µg/ml
21 h: <= 0.5 µg/ml

Test condition : 1g/kg isophorone was administered to two rabbits and the isophorone - blood - concentrations were monitored.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(27)

Type : absorption

Result : Isophorone is absorbed after inhalation of vapors and aerosols and (to a lesser extent) after skin contact.

Reliability : (4) not assignable
No experimental data available.

(31)

Type : Biochemical or cellular interactions

Result : 0.00027 - 114.0 mg/l 3,5,5-trimethylcyclohex-2-enone did not affect respiration of rat liver mitochondria.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(48)

Type : Biochemical or cellular interactions

Remark : The objective of the study was to characterize the ability of [3H]-2,4,4-Trimethyl-2-pentanol (abbreviated as TMP-2-OH)to bind to alpha-2-u in vitro and to determine whether other compounds that cause this protein to accumulate hve the same binding characteristics.

Result : The Ki for isophorone was 7.7x10exp-6 M, while the Ki values for d-limonene, 1,4-dichlorobenzene and 2,5-dichlorophenol were all in the range 10exp-4 M.

Test substance : Isophorone was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(7)

Type : Biochemical or cellular interactions

Method : Groups of 5 male Sprague-Dawley rats weighing 250-300 g were exposed for 4 hrs to either clean filtered air or 19, 49, 67 and 90 ppm Isophorone (vaporized by bubbling). At the end of the exposure period, rats were anaesthetized with pentobarbital and exsanguinated from the abdominal aorta. Blood samples were collected in tubes containing complexon as an anticoagulant. Red blood cells, total white blood cell and leucocyte differential counts were performed.

Result : A statistically significant decrease in the number of

circulating leucocytes (without any change in differential or red blood cell counts) was observed in rats exposed for 4 hrs to increasing concentrations of isophorone.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(10)

Type : Biochemical or cellular interactions

Result : Depletion of glutathione content in
liver: 40 % (maximum: 4 h after application)
testes: 82 % (maximum: 4 h after application)
epididymis: 72 % (maximum: 8 h after application)

Test condition : 500 mg/kg isophorone was i.p. administered to male sexually matured Sprague-Dawley rats and the glutathione levels were measured and compared to the levels of control rats (depletion of glutathione in liver, testes and epididymis).

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(39)

Type : Distribution

Method : Partition coefficients blood:urine, urin/blood, urine/air

Result : Partition coefficients isophorone at 37°C:
urine/air = 710
blood/air = 2349 (highest among all ketones tested)
blood/urine = 3.3

Conclusion:
substances with high blood/air coefficient may be characterized:
- remarkable pulmonary absorption levels
- high concentrations in arterial blood
- risk of high storage in organs
- long periods of permanence in the body

Test condition : The partition coefficients urine/air and blood/air were measured using blood and urine samples from healthy volunteer subjects.
- 20 min equilibration between liquid (10 ml) and air phase (50 ml) at 37 degree C,
- GC analysis of air phase, equilibration with new air phase,
- 11 equilibration cycles,
- determination of partition coefficient from exponential decrease of concentration curve
Urine/blood coefficient was determined.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(72) (73)

Type : Distribution

Result : Blood: 280 µg isophorone/ml blood (rapid diffusion)

1.
Rats
Distribution in organs (no differences between sex and time of death) from high to low amounts:

1. Stomach
2. Pancreas
3. Adrenal
4. Spleen
5a. Ovaries, Tubes
5b. brain, heart, lung, kidney
5c. Testes
6. Liver

Rabbit
1. Stomach
2. Ovaries
3. Adrenal
4. Spleen, liver
5. Kidney, brain, heart
6. Lung

2.
Traces of isophorone in stomach (1-5 µg/g)

Conclusion:
- Rapid diffusion and distribution of isophorone through body
- No bioaccumulation

Test condition : The distribution of isophorone in organism was studied after ingestion.
1.
Animals:
- 3 male Wistar rats
- 3 female Wistar rats
- 1 female New-Zeeland rabbit
Dose: 4 g/kg in olive oil via gavage
Deaths: 3 male rats and the rabbits: 1 h after ingestion
3 female rats: 1, 3, 5 h after ingestion

2.
Animals:
- 3 male and 3 female Wistar rats
Dose: 1 g/kg in olive oil via gavage
Deaths: 48 h after ingestion
Experiments:
- blood sample determination of rabbit (0,5 h after ingestion)
- Extraction of organs and GC analysis (identification of isophorone via Kovats index)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(27)

Type : Distribution

Result : Rats - male and female (only very slight sex differences)
Distribution in organs from high to low amounts:
1. Kidney
2. Pancreas, adrenals, liver, brain

The total amount of isophorone measured in organs decreased with increasing post-exposure period.

Conclusion: rapid diffusion and distribution of isophorone through body

Test condition : The distribution of isophorone in organism was studied after inhalation for 4 h.
Animals:
- 3 male Wistar rats
- 3 female Wistar rats
Dose: 2000 mg/kg (400 ppm)
Deaths: 1 male and 1 female - immediately
1 male and 1 female - 1 h 30 m
1 male and 1 female - 3 h
Experiments:
- Extraction of organs and GC analysis (identification of isophorone via Kovats index)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(27)

Type : Distribution

Result : Measured radioactivity (both sexes and species)
Kidney > liver > lung >> pancreas > adrenals

Test condition : 500 mg 14C-Isophoron/kg were orally administered to B6C3F1 mice and F344 rats and the distribution was measured 24 hours after administration.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(131)

Type : Excretion

Result : 80 % of radioactivity was excreted after 96 h.
50-65% of radioactivity was excreted via urine (single-dose and repeated-dose).
Slight difference between single-dose and repeated-dose administration: single-dose treated rats excrete a slightly higher amount of isophorone via feces compared to repeated-dose treated rats.

Conclusion: Isophorone is rapidly excreted - mainly via glucuronidation. No evidence of bioaccumulation in rats.

Test condition : 500 mg/kg isophorone was administered in a single dose to male rats.
500 mg/kg isophorone was administered to male rats for 8 consecutive days.
Urine, feces and expired air were collected and the amount of radioactivity was determined.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment.

Flag : Critical study for SIDS endpoint
27.06.2003

(131)

Type : Excretion

Result : Expired air:
0-0,5 h: 110 µg

0,5-1 h: 100 µg
1-1,5 h: 40 µg
1,5-2 h: 70 µg
2-2,5 h: 50 µg
2,5-3 h: 30 µg

Test condition : The expiration of isophorone in organism was studied after inhalation for 4 h.
Animals:
- 3 male Wistar rats
- 3 female Wistar rats
Dose: 2000 mg/kg (400 ppm)
Deaths: 1 male and 1 female - immediately
1 male and 1 female - 1 h 30 m
1 male and 1 female - 3 h
Experiments:
- examination of the expired air (2 rats, which were killed 3 h after end of exposure)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

(27)

Type : Metabolism

Remark : No distincion was made between alpha-isophorone, CAS-78-59-1 and beta-isophorone, CAS 471-01-2

Result : Urine:
The following substances were identified both in rabbits and rats urine, however in varying ratios:

Unreacted isophorone
3,5,5-trimethylcyclohexan-1-one (dihydroisophorone - mainly in rats)
3,5,5-trimethyl-2-cyclohexen-1-ol (isophorol - mainly in rabbits) eliminated as glucuronide
3,5,5-trimethylcyclohexan-1-ol (CAS 116-02-9), cis (933-48-2)
and trans (767-54-4) isomers.
These compounds were identified only via GC and correlation to Kovats indices.

Further compounds were seen, but could not be identified.

5,5-dimethyl-2-cyclohexen-1-one-3-carboxylic acid was extracted, isolated and identified (GC, IR) in the urine of rabbits.

Expired air of rats and rabbits:
unreacted isophorone

Conclusion:
The main metabolite of rabbits after isophorone administration is
5,5-dimethyl-2-cyclohexene-1-one-3-carboxylic acid found as glucuronide conjugate in the urine (after 48 hours). Further detoxifications occur in rabbit and rat: Hydrogenation of the 1-one-, the 2-ene- and both positions.

Test condition : TEST ORGANISMS
- Rabbits, New Zealand White
- Rats, Wistar
- Weight at study initiation:

rabbits ca. 2.5 kg; rats ca. 250 g
- Number of animals: not reported

ADMINISTRATION / EXPOSURE
- Duration of test/exposure: single dose
- Type of exposure: gavage
- Post exposure period:
  Air sampling for 6 hours after dosing
  Urine sampling 24 and 48 hours after dosing
- Vehicle:
  rabbits: pure substance followed by ca. 20 ml water
  rats: olive oil
- Concentration in vehicle: 0.2 g/ml (rats)
- Total volume applied: 1 ml/200 g bw (rats)
- Doses: 1000 mg/kg bw

SAMPLING
- Exhaled air (part of the animals): absorption on charcoal
- Urine sampling for 48 hours

ANALYSIS
Urine: Enzymatic hydrolysis by beta-glucuronidase (buffered at pH 4.7, 37 degree C), extraction, gas chromatography, identification using Kovats index
Charcoal: Elution with dichloromethane, gas chromatography, identification using Kovats index

Test substance : origin: Ugine-Kuhlmann
ca. 91.5 % alpha-isophorone, CAS-78-59-1
ca. 8.5 % beta-isophorone, CAS 471-01-2
traces 3,3,5-trimethylcyclohexanone, CAS 873-94-9

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(28)

Type : Metabolism

Result : Tollens reaction was positive. The metabolite was identified as 5,5-dimethyl-1-cyclohexene-3-one-1-carboxylic acid.

Isophorone appears to undergo oxidation at the 3-methyl group and further glucuronidation and excretion via urine.

Test condition : - Application of 1,000 mg/kg bw isophorone to rabbit (gavage)
- Collection of complete urine for 4 consecutive days
- Acidification of urine with hydrochlorid acid (pH 1-2)
- Performance of Tollens reaction to determine glucurono-conjugates.
- Extraction with diethyl ether for 48 hours
- Drying with Na2SO4
- Complete removal of solvent in vacuo
- reflux for 10 min in hexane / chloroform (2:1 v/v)
- withdrawal of solvent
- recrystallisation from boiling water
- characterisation by:
  melting point (158 degree C)
  mass spectrometry (168 g/mole)
  UV, IR, NMR spectrometry
  optical activity: no rotation
  determination of elements (C: 64.46 %; H: 7.22 %)

Test substance	:	commercial (Prolabo), "of limited purity"
Conclusion	:	The 3-methyl group of isophorone is oxidized leading to a carboxylic acid group.
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(136)

Type	:	Metabolism
Result	:	Determined metabolites in the urine of male rats after isophorone ingestion were: - 6-Oxoisophoron (3,5,5-Trimethyl-2-cyclohexen-1,6-dion), unreacted isophorone, dihydroisophoron, 4-oxoisophoron (3,5,5-Trimethyl-2-cyclohexen-1,4-dion), 4-hydroxyisophoron (4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1,4-dion), 6-hydroxyisophoron (6-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-on) In contrast to the findings of Dutertre-Catella, 1978, the alcohols isophorol and 3,5,5-trimethylcyclohexan-1-ol were not found.
Test condition	:	500 mg/kg isophorone was administered to male rats for 8 consecutive days, the urine was collected and the metabolites were determined.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

(131)

Type	:	other: DNA-binding study
Result	:	Significant attachment of radioactivity to DNA could not be found.
Test condition	:	- rats: F344, male/female, 5 per group - mice: B6C3F1, male/female, 25 per group - single administration of 500 mg/kg unlabelled isophorone spiked with 0.4 mCi per rat and 0.08 mCi per mouse; - control: only rats, only radioactive substance - vehicle: neutral oil - sacrifice and removal of liver and kidneys after 24 hours - further processing individually for rats / pooled for five mice - preparation of cell nuclei - isolation of DNA - liquid scintillation counting
Test substance	:	unlabelled substance: Aldrich [1,3,5-14C]isophorone: Amersham-Buchler, 52 mCi/mol
Conclusion	:	Neither isophorone nor its metabolites are covalently bound to DNA. Degradation products are also not incorporated into newly formed DNA. The carcinogenicity observed in the NTP study is probably not caused by genotoxic effects of isophorone.
Reliability	:	(1) valid without restriction Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

(132)

Type : other: Nephrotoxicity

Result : Treatment of male Sprague-Dawley rat with isophorone (150 mg/kg) by gavage for 14 consecutive days resulted in a significant intensification of a protein band corresponding to kidney-type-alpha 2u-globulin.

Test condition : The effects of alpha 2u-globulin accumulating agents on alpha 2u-globulins in rat kidneys were examined by SDS-PAGE and immunoblotting analysis.

Test substance : purity > 97 %

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

(111)

Type : other: Nephrotoxicity

Remark : The NCI-Black-Reiter (NBR) rat is the only strain of male rat known not to synthesize the hepatic form of the low molecular weight protein alpha 2u-globulin.

Result : Under exposure conditions that clearly induce alpha 2u-nephropathy in male F344 rats, no lesions, hyaline droplets, or alpha 2u-globulin were detectable in treated or control male NBR and female F344 rats.

It is thus concluded that the presence of alpha 2u-globulin is causal to the development of renal disease in rats exposed to isophorone.

Test condition : The objective of this study was to show that the presence of alpha 2u-globulin is essential for the development of nephropathy in rats exposed to isophorone.

Positive control: Induction of alpha 2u-nephropathy in F344 male rats with lindane; response was contrasted to male NBR and female F344 rats.

Negative control: NBR male and F344 male and female rats gavaged with corn oil served as negative controls.

Experiment: 5-7 male NBR rats (age 11 weeks) were exposed to isophorone at 1000 mg/kg/day and male and 5 female F344 rats (age 11 weeks) were exposed to lindane (10 mg/kg/day) by oral gavage on 4 consecutive days.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(26)

Type : other: Nephrotoxicity

Result : Under the incubation conditions used, 30% of native alpha 2u-globulin was degraded in a 4 hr-period. Binding of isophorone to alpha 2u-globulin decreased alpha 2u-globulin degradation by 33%, suggesting that a decrease in lysosomal degradation is involved in the accumulation of this protein in male rat kidney lysosomes.

Test condition : It is suggested, that isophorone causes a male rat-specific nephrotoxicity via binding to a2u-globulin and resulting in

accumulation of a2u-globulin in renal lysosomes. It is supposed, that isophorone decreases its degradation by lysosomal proteinases.
The lysosomal degradation of native alpha 2u-globulin and that to which isophorone was bound was studied.
Alpha2u-globulin was purified from male rat urine, and male rat renal cortical lysosomes, isolated by differential centrifugation, served as the proteolytic enzyme source.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail.

Flag : Critical study for SIDS endpoint

(80)

Type : other: Nephrotoxicity

Result : Isophorone forms complexes with a2u-globuline.

Test condition : Mechanism of <alpha>2u-globulin nephropathy:
30-50 mg/d of a2u-g is synthesized in the liver of male rats, transported to the kidney and then filtered.
- 60% is reabsorbed by kidney
- 40% remains in filtrate and is excreted into urine
= a2u-g - major urinary protein in male rats (all other filtered proteins are completely reabsorbed)

a2u-g content
- female rats: 120 times lower
- mice: nearly absent
- human: nearly absent

Chemicals bind reversibally to a2u-g in the liver of animals - protein complexes are less digestible (difficult to hydrolyse) - protein complexes accumulate in the form of polyangular droplets - nephropathy

It is studied, whether isophorone forms such a2u-globulin complexes.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

(18) (126)

Type : other: Nephrotoxicity

Result : 1. Protein droplet formation was increased after isophorone treatment
2. Isophorone and dihydroisophorone was identified after treatment with isophorone and dihydroisophorone respectively. Isophorone was also identified in samples from animals treated with isophorol.

Conclusion: Isophorone as well as the supposed metabolites isophorol and dihydroisophorone may induce a2u-globulin-nephropathy

Test condition : 1. Male rats were treated with 0.5 or 1.0 g/kg isophorone and the amount of protein droplets was measured.
2. Male rats were treated with isophorone and its metabolites isophorol and dihydroisophorone. 24 h after treatment a2u was isolated from male rat kidney cytosol.
GC/MS was performed on a2u samples.

Reliability : (4) not assignable

Documentation insufficient for assessment.

Flag : Critical study for SIDS endpoint

(123)

Type : other: Neurobehavioural effects

Result : Median active level causing 50 % decrease of duration of immobility in the mouse 'behavioural despair' swimming test, ID50 = 0.63 mg/l.

Test condition : - Test organism: Male Swiss OF1 mice
- Inhalation exposure time: 4 h

Test substance : 96 % pure

Reliability : (4) not assignable
Documentation insufficient for assessment

(21)

Type : other: Sensory irritation

Result : The responses obtained for various concentrations were utilized to develop a concentration-response relationship. From this relationship, the concentration associated with a 50 % decrease in respiratory rate, RD50, was calculated.
Predicted effect levels in humans:
- intolerable = RD50: 27.8 ppm = 160 mg/m3
- uncomfortable = 0.1 * RD50: 3 ppm (16 mg/m3)
- minimal/no effect = 0.01 * RD50: 0.3 ppm (1.6 mg/m3)

Test condition : Test organism:
- Strain, sex: male Swiss OF1 mice
- weight: 25 +/- 2 g
- number of animals: 6 per concentration

- Exposure: inhalation, 5 minutes
- at least 4 different concentrations
- Endpoint: reflex decrease in respiratory rate

Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment

(22)

Type : other: Upper-Respiratory-Tract Irritation

Result : Median active level causing 50 % decrease of the respiratory rate, RD50 = 0.16 mg/l

Test condition : Test organism:
- Strain, sex: male Swiss OF1 mice
- weight: 20-25 g
- number of animals: 6 per concentration

- Exposure: inhalation, 15 minutes
- at least 4 different concentrations
- Endpoint: breathing frequency

Test substance : 96 % pure

Reliability : (4) not assignable
Documentation insufficient for assessment

(21)

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