FOREWORD INTRODUCTION

3-Methyl-2-butenal

CAS N°:107-86-8

SIDS Initial Assessment Report

For

SIAM 17

11–14 November 2003, Arona, Italy

1.	**Chemical Name:**	3-Methyl-2-butenal
2.	**CAS Number:**	107-86-8
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:**	BASF AG, Germany; KURARAY CO., LTD., Japan.
5.	**Roles/Responsibilities of the Partners:**	
•	Name of industry sponsor /consortium	BASF AG, Germany Contact person: Dr. Hubert Lendle, GUP/CL - Z570 D-67056 Ludwigshafen
•	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): 10 March 2003 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 30 April 2003 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms
8.	**Quality check process:**	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9.	**Date of Submission:**	August 13, 2003
10.	**Date of last Update:**	

11. Comments:

OECD/ICCA - The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

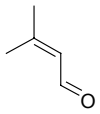
- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD 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 o f 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

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	107-86-8
Chemical Name	3-methyl-2-butenal
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

No specific studies are available regarding toxicokinetics and metabolism of the substance.
3-Methyl-2-butenal is of moderate acute oral and acute inhalation toxicity and of low acute dermal toxicity.
LD_{50} rat (oral): 690 mg/kg bw; symptoms: labored breathing, stagger, tremors, apathy.
LC_{50} rat (inhalation): 3,700 mg/m³/4h (vapor); symptoms: lacrimation, nasal discharges, closed lids, labored breathing, dyspnoea, stagger, tremors, apathy.
LD_{50} rabbit (dermal): 3,400 mg/kg bw; symptoms: dyspnoea, stagger, abdominal position, apathy, local skin irritation.
Undiluted 3-methyl-2-butenal is corrosive to the skin and highly irritating to the eyes. Vapors of the material are irritating to the skin, the eyes, and the respiratory tract. In a well documented guinea pig maximization test, 3-methyl-2-butenal showed a skin sensitizing potential.

In a 28-day inhalation study (OECD TG 412) with rats, the NOAEC for systemic toxicity was at the highest tested concentration of 300 ppm (1,020 mg/m^3, approx. 252 mg/kg bw/day). Symptoms of irritancy were seen in the respiratory tract at 100 ppm in terms of focal metaplasia of respiratory epithelium of the nasal cavity into squamous epithelium, changes in cartilage of larynx, and in some cases atrophy of the olfactory epithelium at 300 ppm. Purulent rhinitis in all males and some females was seen at 300 ppm. This was regarded to be responsible for reduced body weight development in both sexes (-59.8% in males and –39.7% in females compared to controls) and impaired terminal body weight in the high dose males (-14.1%), rather than systemic toxicity. There were no other findings which would indicate systemic toxicity. The NOAEC for local irritation was determined to be 30 ppm (100 mg/m^3, approx. 25 mg/kg bw/day), the LOAEC was 100 ppm (340 mg/m^3, approx 84 mg/kg bw/day) due to the histopathological changes observed in the respiratory tract.
As part of a one generation study in rats (OECD TG 415), 3-methyl-2-butenal was administered subchronically at concentrations ranging from 50 - 800 ppm (about 6 – 77 mg/kg bw/day) via the drinking water for a period of 18 weeks. The highest test concentration (about 77 mg/kg bw/day) did not lead to any adverse findings in both sexes of the F0 parental animals. No higher dose levels were achievable using drinking water due to palability problems that were already noted at the highest tested concentration.

In vitro genotoxicity studies (Ames test) failed to produce coherent results. 3-Methyl-2-butenal revealed no mutagenic effect in the classical Ames test whereas positive effects were reported from a Liquid Suspension Assay in the strain TA 100 with and without metabolic activation. In the mouse micronucleus test (OECD TG 474) the material was not clastogenic nor did it impair chromosome distribution during mitosis. No unscheduled DNA repair was induced in rat liver cells by the test material or metabolites in an *in vivo* test system according to OECD TG 486.

In a one-generation study (OECD TG 415) in rats with administration of 3-methyl-2-butenal in the drinking water, no impairment for reproductive performance and fertility, systemic toxicity and developmental toxicity was found at the highest tested dose of 800 ppm (about 77 mg/kg/day) thus representing the NOAEL for the F0 parental animals and their progeny.
In a prenatal toxicity study (OECD TG 414), 3-methyl-2-butenal had no effects on the gestational parameters and induced no signs of embryo- and fetotoxicity, in particular no indications for teratogenicity. The NOAEL for maternal toxicity and prenatal developmental toxicity was found at 300 mg/kg bw/day. A higher dose (450 mg/kg bw/day) could not be evaluated since it lead to severe toxicity or was lethal for the dams.

Environment

3-Methyl-2-butenal is a colorless-faint yellowish liquid with a water solubility of 110 g/l at 20°C. The vapor pressure is 7.5 hPa at 20°C. The melting point is lower than –20°C (calculated value –76.8°C) and the boiling point is 136°C. 3-Methyl-2-butenal has a density of 0.876g/cm³ at 20°C. The flash point is at 37°C and therefore, the substance is flammable.
The calculated Henry´s Law Constant based on measured data for water solubility and vapor pressure is 0.57 Pa*m³/mole, otherwise using the EPIWIN calculation program a constant of 2.85 Pa*m^3/mole could be calculated. Due to the measured log K_{ow} of 0.53 at 25 °C and the calculated log K_{oc} of 0.9 (Koc = 7.9), bio- and geoaccumulation are not to be expected. Distribution modeling using Mackay Level I indicates water (83%) and air (17%) to be the main target compartments. 3-Methyl-2-butenal is readily biodegradable according to OECD evaluation criteria (CO_2 –Headspace Test; ISO 14593, 80-90 % after 28 d) and the 10-days time window was fulfilled. Hydrolysis at environmental pH conditions is not expected according to the structure. In the atmosphere, the substance will be indirectly photodegraded by reaction with OH-radicals (calculated $t_{½}$ = 8.2 h) or ozone (calculated $t_{½}$ = 23.2 h).
The acute aquatic toxicity has been determined for fish (*Leuciscus idus* LC_{50} (96h) 17.6 mg/l), invertebrates (*Daphnia magna* EC_{50} (48h) 13.5 mg/l) and green algae (*Scenedesmus subspicatus* ErC_{50} (72h) 22.4mg/l, EbC_{50} (72 h) 16.7 mg/l). Due to a moderate volatility the LC_{50} for fish was corrected and therefore, the effective LC_{50} (96h) was 12.7 mg/l. Prolonged or chronic studies are not available. Based on the most sensitive data, LC_{50} (96h) 12.7 mg/L (corrected), a $PNEC_{aqua}$ of approximately 13 µg/l can be derived by applying an assessment factor of 1,000, according to the Technical Guidance Document for the EU risk assessment procedure.

Exposure

The worldwide production volume of 3-methyl-2-butenal was 6,000 - 13,000 metric tonnes in 2001. 3-Methyl-2-butenal is mainly used as an intermediate (99 %) in closed systems for chemical synthesis and only 1% are sold to German clients as well as exported to Switzerland.
The substance is used internally and further processed to citral and vitamin A. Worker protection is adequate and includes the use of appropriate technical equipment during substance handling, monitoring of concentrations in the air at the work place, the use of protective equipment, etc. However, the risk of exposure to 3-methyl-2-butenal may exist after spillages and during accidental exposure.
An estimated volume of < 10 t/a is used for reconstitution of essential oils and other natural products.
Consumer exposure is negligible since only small amounts of the 3-methyl-2-butenal are contained in food at maximum concentrations of 50 ppm. The substance naturally occurs in several plants, e.g. in blackberries (up to 0.34 %) and wild ginger (0.05 %). Further, the substance was detected in raw meat (0.36 %) and barbecued chicken (9 µg/kg). Also, exposure of the general population via the environment is negligible. 3-Methyl-2-butenal was listed in the Swiss Product Register (2002) as to be found under the collective term “aromas, perfumes” in 9720 products without any information about the concentrations of the substance. How many of these products indeed contain 3-methyl-2-butenal cannot be resolved. The substance is not contained in products registered in Denmark, Sweden, Finland or Norway.
Releases into the environment may occur during production and processing of 3-methyl-2-butenal as intermediate as well as from direct use of the substance and from formulation and use of products containing it.
During production and processing at the sponsor company the effluent concentration of the sewage treatment plant was below 20 µg/l. Less then 25 kg/a were emitted into the air.

RECOMMENDATION

The substance is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Environment:

The chemical is currently of low priority for further work. 3-Methyl-2-butenal possesses properties indicating a hazard for the environment. Although these hazards do not warrant further work as they are related to acute toxicity, which may become evident only at very high exposure levels, they should nevertheless be noted by chemical safety professionals and users.

Human Health:

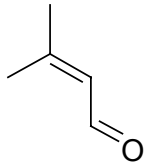
The chemical possesses corrosive and sensitizing properties indicating a hazard for human health. Given the main use as a chemical intermediate in closed systems and the very low content of the substance in consumer products in the Sponsor country, the substance is considered to be of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 107-86-8
IUPAC Name: 3-methyl-2-butenal
Molecular Formula: C5H8O
Structural Formula:



Molecular Mass: 84.12 g/mole
Synonyms: 3-methylbuten-2-al-1
3-methylcrotonaldehyde
3,3-dimethylacrolein
.beta.-methylcrotonaldehyde
.beta.,.beta-dimethylacrylic aldehyde
.beta.,.beta.-dimethylacrolein
Seneci(o)aldehyde
Prenal

1.2 Purity/Impurities/Additives

Substance type:	organic	
Physical status:	liquid	
Purity:	≥ 96 % w/w	
Possible impurities:	3-methyl-3-butenal:	≤ 1 % (w/w)
	3-methyl-3-butanol:	≤ 0.3 % (w/w)
	3-methyl-3-buten-1-ol:	< 1 % (w/w)
	3-methyl-2-buten-1-ol:	< 1 % (w/w)
	3-methylformate:	≤ 0.1 % (w/w)
	formaldehyde:	≤ 0.02 % (w/w)
Form:	liquid	
Color:	colorless-faint yellowish	
Odor:	pungent	

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Melting point	below -20°C* -76.8°C (calculated)	BASF AG, 2001a BASF AG, 2002a
Boiling point	135.9°C (calculated from measured values for vapor pressure)	BASF AG, 1986
Flash point	37°C (flammable)*	BASF AG, 2001a
Explosion limits	lower: 1.8 Vol%* upper: 7.0 Vol%*	BASF AG, 2001a
Ignition temperature	145°C*	BASF AG, 2001a
Vapour pressure	7.5 hPa at 20°C (measured)	BASF AG, 1986
Density	0.876 g/cm³ at 20°C* Flotation or stratification processes in surface waters in case of accident losses are possible.	BASF AG, 2001a
Viscosity	1 cp at 20°C*	BASF AG, 2001a
Water solubility	110 g/l at 20°C (measured)	BASF AG, 1973
Log K_{ow}	0.53 at 25°C (measured)	BASF AG, 1988a
Henry's Law Constant	2.85 Pa*m³/mole at 25°C (calculated; group estimates) 0.57 Pa*m³/mole at 20°C (calculated from measured values)	BASF AG, 2002b according to Thomas, 1990
K_{oc} **Log K_{oc}**	7.9 (calculated) 0.9 (calculated)	BASF AG, 2002c

* manufacturer data without proof

2 GENERAL INFORMATION ON EXPOSURE

The world production of 3-methyl-2-butenal in the year 2001 was in the range of 6,000 to 13,000 metric tons, the production ranges for Europe (1 producer) and Asia (1 producer) are 5,000 to 10,000 and 1,000 to 3,000 metric t/a respectively. In 2001, the substance was not produced in the US.

3-Methyl-2-butenal can be obtained by using isobutene and formaldehyde followed by an oxidative dehydration (BUA, 1998). Further, 2-alkenals can be obtained by the reaction of unsaturated alkyl halides with the sodium salts of secondary nitrohydrocarbons. Another possibility is the treatment of acetals with vinyl ethers in the presence of boron trifluoride to give the corresponding β-alkoxyacetals. Under influence of acids these are converted into α,β-unsaturated aldehyds (Ullmann, 2000).

At BASF AG (sole production site in Europe), production and processing of 3-methyl-2-butenal takes place in closed systems. At BASF AG, 3-methyl-2-butenal is almost exclusively processed (ca. 99%) to citral, which serves as a fragrance and flavor for lemon aromas and as starting material for the synthesis of e.g. vitamin A. The remaining amount of 1% is directly filled into an ISO-container by using special filling equipments. In 2002, 3-methyl-2-butenal was sold to industrial

clients in Germany and exported to Switzerland, respectively (BASF AG, 2003a). It is then used as an aroma chemical and/or flavor as well as for their synthesis.

3-Methyl-2-butenal is used in small quantities (Europe: 27 kg in 1995; USA: 1982: 0.1 kg; 1987: 3 kg; 1995: 0.0 kg), as food flavor in amounts ranging from 0.5 - 7.25 ppm in baked goods and non-alcoholic beverages and up to 10 -50 ppm in chewing gums (RIFM-FEMA Database, 2002). Further, it is used in confection frosting, frozen dairy, fruit ice, gelatin pudding, hard and soft candy and jam jelly (≤ 12.5 ppm). 3-Methyl-2-butenal itself is not listed in the Swiss Product Register (2002). However, it may be contained in 9720 products registered under the collective term "aromas, perfumes" without any information about the concentration of the substance. How many of these products indeed contain 3-methyl-2-butenal cannot be resolved. There is the possibility that some of these products might represent 3-methyl-2-butenal. It is also possible that none of these products do contain this substance. To summarize, 3-methyl-2-butenal itself is not registered in Switzerland (Bormann, 2003). The substance is not contained in products registered in Denmark, Sweden, Finland or Norway.

The Flavor and Extract Manufacturers' Association states that the substance is Generally Recognized as Safe (GRAS) as a flavor ingredient and in a recent publication of the Joint FAO/WHO Expert Committee on Food Additives it was concluded that there are no safety concerns for 3-methyl-2-butenal based on the current intake (RIFM-FEMA Database, 2002 and JECFA, 2003).

An estimated volume of < 10 t/a is used for reconstitution of essential oils and other natural products.

Releases into the environment may occur during production and processing of 3-methyl-2-butenal as an intermediate, as well as from the use of the substance or products containing it.

3-Methyl-2-butenal is measured in the influent and the effluent of the waste water treatment plant of BASF AG, Ludwigshafen (Germany) at regular intervals (24 h-mixing samples). Between 1st January and 12th November 2002 the concentration in the influent as well as in the effluent was always found to be below the limit of quantification (influent: 0.5 mg/l: effluent: 0.02 mg/l; BASF AG, 2002d).

Via production and further processing in 2000 less than 25 kg of 3-methyl-2-butenal were emitted into the air according to the German Emission Register at BASF AG in Ludwigshafen (Germany)(BASF AG, 2002e).

Emission data from other production and processing sites are not available.

2.1 Environmental Exposure and Fate

2.1.1 Sources of Environmental Exposure

Releases into the environment may occur during production and further processing of 3-methyl-2-butenal. Distribution modeling using Mackay Level I V2.11 indicates water to be the main target compartment with 83 % (for input parameters see IUCLID). About 17 % of the substance will be distributed into the air (BASF AG, 2002f). In the air the substance will be rapidly degraded according to the calculated $t_{1/2}$ of about 8.2 hours for OH-radicals and 23.2 hours for ozone molecules using the model AOP v1.90 (BASF AG, 2002g).

3-Methyl-2-butenal is readily biodegradable according to OECD-criteria (80 - 90 % biodegradation within 28 days; criteria of 10-days time window fulfilled) in a CO_2-HeadspaceTest conducted according to ISO 14593 (BASF AG, 2002h). After 3 days and 10 days the substance was degraded

up to 29 % and 75 %, respectively. Therefore, the 10-days time window as a measure for the ready biodegradability was fulfilled. Hydrolysis at environmental pH conditions is not to be expected according to the chemical structure.

The estimated logK_{oc} using the PCKOCWIN model was 0.9; (Koc = 7.9)(BASF AG, 2002c). This indicates that 3-methyl-2-butenal will not adsorb on soil and sediments or suspended solids. The calculated Henry's Law Constant using the vapor pressure, water solubility and molecular weight as input parameter according to Thomas (1990) could be calculated to be 0.57 Pa*m³/mole, whereas the HENRYWIN model calculated a higher constant of 2.85 Pa*m³/mole (BASF AG, 2002 b).

No measured data on bioaccumulation are available. Based on the partition coefficient octanol-water (measured log K_{ow} = 0.53), and the BCF estimation using the equation cited in the TGD: log BCF = 0.85*log K_{ow} – 0.70 a BCF of 0.56 was derived and therefore, bioaccumulation is not expected in aquatic organisms.

3-Methyl-2-butenal occurs naturally as biogenic organic compound up to 0.05 % in root material of wild ginger (Motto and Secord, 1985) and cultivated as well as wild varieties of blackberries up to 0.34 % (Georgilopoulos and Gallois, 1987). Further, it was with 0.36 % detected in raw beef (King et al., 1993) and in dripping fat of roasting chicken with a concentration of 9 µg/kg (Nouleau and Toulemonde, 1987).

2.2 Human Exposure

2.2.1 Occupational Exposure

Exposure may occur during manufacture, transportation and industrial use. The likely primary routes of human exposure to 3-methyl-2-butenal are skin contact and inhalation at the work place. Worker exposure is limited by enclosed systems, industrial hygiene controls and personal protective measures. At BASF AG in Ludwigshafen (Germany) during the last 5 years only 2 occupational exposure measurements had been performed in the production plant for 3-methyl-2-butenal. In both cases the concentrations were found to be below the detection limit (< 0.005 and < 0.007 mg/m^3). The monitoring was carried out under typical work conditions by personal air sampling, giving an 8 hour shift value. The test method involved collecting a defined air volume by using a pump, adsorption of any present substances on a glass tube, filled with charcoal, and subsequent gaschromatographic analysis after desorption.

2.2.2 Consumer Exposure

As a result of further processing to citral and vitamin A, end-use products contain only trace levels of 3-methyl-2-butenal (< 0.01 %) and consumers are generally not exposed to this chemical in the finished products (BUA, 1998). Estimates of human exposure were based on the fact that 3-methyl-2-butenal is added in maximum amounts of 50 ppm as flavor to food. It was estimated that this corresponds to a daily intake of ca. 2 ng/kg bw in a 60-kg person (US Department of Commerce, 1979). 3-Methyl-2-butenal may also be found in food, originating either from the natural occurrence at low concentrations in some plants such as wild ginger root (< 0.05 % of steam-volatile components), blackberries (0.2 - 1.86 % of aldehydes detected in juice), and capers, or originating from formation during food processing due to oxidative deamination or decarboxylation of aminoacids (Strecker degradation) or reactions between amino acids and carbohydrates (Maillard reactions). In barbecued chicken fat content of 3-methyl-2-butenal was 9 µg/kg (Nouleau and Toulemonde, 1987).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No specific studies are available concerning kinetic or metabolic fate of the substance. Based on the acute studies, it can be concluded that the substance can be absorbed by the oral, dermal and inhalative route.

3.1.2 Acute Toxicity

The acute toxicity of 3-methyl-2-butenal was investigated in a number of non-guideline studies. However, as the methods used are similar to current OECD test guidelines and the results are well documented, the studies can be fully evaluated.

Oral

The acute oral toxicity is moderate: The LD_{50} in rats after a post dosing observation period of 14 days was determined to be ca. 690 mg/kg bw (BASF AG, 1979a). The symptoms were described as apathy, dyspnoea, salivation (at 316 mg/kg bw or higher), labored breathing, tremors, unsteady gait (at 464 mg/kg bw or higher), reddening of skin, unkempt fur, lacrimation (at 562 mg/kg bw or higher) and spasms (at 1,000 mg/kg bw).

The acute oral LD_{50} for a product with technical purity (about 80% 3-methyl-2-butenal and 20 % 3-methylbut-3-en-1-ol) was reported as 810 mg/kg bw for rats after a 7 days post dosing observation period (BASF, 1971).

Inhalation

The acute inhalation toxicity is moderate: the combined LC_{50} for male and female rats was 3.7 mg/l/4 hours (vapor), corresponding to 3,700 mg/m³/4 hours (BASF AG, 1979b). Short term exposure of rats to saturated or highly enriched atmospheres (ca. 21 – 24 mg/l, corresponding to 21,000 - 24,000 mg/m³) caused mortality in 1/6 and 6/6 animals within few hours or days after 30 and 60 min. of exposure, but no deaths in a group of 12 rats after 10 min. inhalation (BASF AG, 1979a). At low concentrations, clinical signs included lacrimation, nasal discharges, closed lids and labored breathing. At higher concentrations, the signs were more pronounced and included also dyspnoea, staggering, tremors, apathy, and opacity of cornea.

In a second Inhalation Risk Test with a product of technical purity (about 80 % 3-methyl-2-butenal and 20% 3-methylbut-3-en-1-ol) concentrations of about 28 – 44 mg/l (corresponding to 28,000 - 44,000 mg/m³) caused no mortality in a group of 12 rats after 10 min. of exposure, but was lethal for all animals (6 per group) after 30 and 60 min., respectively (BASF, 1971). As clinical signs mainly eye irritation and dyspnoea were described.

Dermal

The acute dermal toxicity is low: dermal LD_{50} in rats was 3,400 mg/kg bw (BASF AG, 1979a). Clinical signs were skin irritation, staggering, abdominal position, dyspnoea, apathy.

Conclusion

3-Methyl-2-butenal was found to be of moderate acute toxicity after oral ingestion and inhalation and of low acute toxicity after dermal application. The oral LD_{50} for the rat was 690 mg/kg bw and

after dermal exposure 3,400 mg/kg bw. The inhalative LC_{50} was reported as 3.7 mg/l/4hrs for rats (3,700 mg/m^3/4hrs). During inhalation exposure the clinical signs indicated irritation of the respiratory tract and of the eyes.

3.1.3 Irritation

Skin Irritation

3-Methyl-2-butenal was corrosive to the skin of rabbits after 1 and 4 hours of exposure and caused necrosis which was confirmed during the pathological examination of the skin (BASF AG, 1979a). Based on these results no more eye irritation test was performed.

Eye Irritation

In the eyes of rabbits, 3-methyl-2-butenal (purity 80 % 3-methyl-2-butenal and 20 % 3-methylbut-3-en-1-ol) caused persistent moderate edema and erythema, persistent corneal opacity, and staphyloma when applied undiluted at a volume of 0.05 ml. The changes were not completely reversible within a post observation period of 8 days (BASF AG, 1971).

Conclusion

3-Methyl-2-butenal was corrosive to the skin of rabbits. In the rabbit eye, the substance was severely irritating and caused persistent opacity. Contact with skin and eye bears the risk of serious damage to the skin and the eyes.

3.1.4 Sensitisation

In a Guinea pig maximization test under GLP according to Directive EEC 84/449 B6 3-methyl-2-butenal was positive when it produced positive skin reactions upon challenge in 3/9 test animals (BASF AG, 1991a).

Conclusion

3-Methyl-2-butenal showed a skin sensitizing potential in the Guinea pig maximization test.

3.1.5 Repeated Dose Toxicity

Inhalation

A subacute 28-day inhalation toxicity study with 3-methyl-2-butenal was conducted under GLP conditions and according to OECD TG 412. Rats were exposed 5 days/week, 6 hours each, to 0, 30, 100, and 300 ppm, corresponding to 0, 100, 340, and 1,020 mg/m³ (BASF AG, 1994). These concentrations can be converted to dosages of 0, 25, 84 and 252 mg/kg bw/day (minute ventilation volume: 240 ml, mean body weight: 0.350 kg). 5 animals per sex and concentration were used.

At 30 ppm (100 mg/m³), signs of sensory irritation, e.g. snout wiping, eyelid closure, were seen at the beginning of the exposure period. However, animals accustomed to the treatment within few days and no other substance-related effects were seen. Substance related effects were seen at the high and mid dose level. In 5/5 male and 3/5 female animals exposed to 100 ppm histopathology revealed focal metaplasia of respiratory epithelium into squamous epithelium, and changes were also seen in larynx cartilage of two males and one female. In animals exposed to 300 ppm, signs of irritancy were more pronounced. Histopathology revealed purulent inflammation in the nasal cavity of all males and some females, focal metaplasia of respiratory epithelium into squamous epithelium in the nasal cavity and in larynx of all males and site specifically in all or some females, and

atrophy of the olfactory epithelium in 1/5 male and 1/5 female. An increase in the number of polymorphonuclear neutrophils and a decrease in leucocytes was interpreted as being associated with the described respiratory tract inflammations. No systemic toxicity was noted as the observation of impaired body weight gain (-59.8 % in males and -39.7 % in females compared to controls from days 0 – 28), and reduced terminal body weights in all high dose males (mean -14.1 %) and some of the high dose females (mean -7.2 %) were attributed to the general discomfort resulting from the purulent inflammation.

A substance related effect for the significantly decreased absolute testes weights in the males treated with 100 ppm and 300 ppm and the significantly increased relative testes weights at 300 ppm was regarded as unlikely as there were no histopathological correlates in this organ. At least for dose group 3 (300 ppm), the weight changes seemed to be a consequence of the reduction of the terminal body weight. There were no other treatment related significant changes in clinical examinations, opthalmoscopy, clinical chemistry, hematology, urinalyses, organ weights, gross lesions, or microscopical examinations. No ophthalmologic changes were detected.

Based on these findings, the NOAEC for irritant effects was 30 ppm (100 mg/m³, approx. 25 mg/kg bw/day) and LOAEC for irritancy was 100 ppm (340 mg/m³, approx. 84 mg/kg bw/day). Since no signs of systemic toxicity occurred, the NOAEC for systemic toxicity was 300 ppm (1,020 mg/m³, approx. 252 mg/kg bw/day).

Oral

Oral toxicity information was recently gained in a reproduction toxicity study conducted according to the OECD TG 415 under GLP conditions (see also chapter 3.1.7, Reproduction / Developmental Toxicity). 25 male and 15 female rats were exposed to 3-methyl-2-butenal via the drinking water containing 50, 200, and 800 ppm of the test article (BASF AG, 2002j). This resulted in mean test substance uptake of ca. 6, 21, and 77 mg/kg bw/day in both sexes over an administration period of 18 weeks. No substance-related signs of toxicity were noted at any dose level. Body weight and body weight gain were not affected. Gross pathology at terminal examination did not indicate increased incidences of lesions or a specific target organ. Histopathology of the reproductive organs did not reveal any adverse effect at any dose. Water and food intake was unchanged at the low and intermediate dose level. Water intake was significantly reduced in both sexes (males ca. 24 %, females ca. 20 %) over the entire treatment period whereas food intake was significantly reduced in high dose males only during the first four weeks of treatment.

Reduced consumption of food and water in the high dose animals was regarded as a substance-related effect, most likely due to the intensive taste and odour of the test substance, but not as an adverse effect (NOEL 21 mg/kg bw/day). Therefore, no adverse effect was noted during subchronic exposure up to and including the highest tested dose of 77 mg/kg bw/day (NOAEL).

Conclusion

After subacute inhalation exposure to 3-methyl-2-butenal no treatment-related findings were noted at 30 ppm (100 mg/m³; NOAEC). Local irritant effects were seen at 100 ppm (340 mg/m³) and above (LOAEC). Purulent inflammation of the upper respiratory tract resulted from the irritancy and caused reduced body weight gains and terminal body weights in both sexes via general discomfort and reduced food intake, rather than systemic toxicity. Due to irritancy, histopathological changes of respiratory epithelium and larynx were seen at 100 and 300 ppm (340 and 1,020 mg/m³); and atrophy of the olfactory epithelium was additionally seen at 300 ppm. For systemic toxicity the NOAEC was 300 ppm (1,020 mg/m³).

No treatment-related findings were noted in rats after oral subchronic exposure for 18 weeks in both sexes up to 77 mg/kg bw/day. Via drinking water no higher doses than approx. 80 mg/kg bw/day can be administered due to palatability problems.

3.1.6 Mutagenicity

In vitro Studies

3-Methyl-2-butenal was tested negative in the Ames test at doses of up to 2,500 µg/plate in Salmonella strains TA1535, TA100, TA1537, TA 1538, and TA98 with and without metabolic activation. No cytotoxicity was noted at the highest concentration (BASF AG, 1979c). An additional Salmonella mutagenicity test similar to OECD TG 471 (Ames preincubation test, Liquid suspension assay, concentration up to 6,000 µg/plate) was carried out with Salmonella strains TA100 and TA98. In the latter study, three experiments were conducted due to equivocal results. Cytotoxicity was noted with TA 100 only at 1,000 - 3,000 µg/plate. Positive results were obtained with TA 100 with and without metabolic activation at concentrations of 2,500 – 5,000 µg/plate in the first experiment (2.1 - 2.5 fold increase in the number of revertants), and more clearly in the 2nd and also at 100 - 500 µg/plate in the 3rd experiment (1.7 - 8.1 fold increase). The slight increase in TA 98 in the first experiment was not concentration dependent (2.1 fold increase). Thus, the positive outcome of the latter study is ambiguous since the results were not consistent between the three experiments and the dose-relationship was not clear (BASF AG, 1991b).

Insufficiently documented studies with bacterial test systems (SOS-Chromotest in E. coli PQ37 or PM21 and Ames test with S. typhimurium TA 100) were reported both as negative or ambiguous (Eder et al., 1990; Eder et al., 1993).

In in vitro experiments, 3-methyl-2-butenal was found to form adducts with deoxyguanosine and 2'-deoxyguanosine-5'-monophosphate, but not with any other nucleosides or nucleotides (Eder and Hoffman, 1993).

In vivo Studies

3-Methyl-2-butenal was tested for its ability to induce micronuclei in mice bone marrow erythrocytes at oral doses up to 700 mg/kg bw/day under OECD TG 474 and GLP conditions (BASF AG, 1992). These dosages led to evident signs of toxicity. The test substance did not have a chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells in vivo.

3-methyl-2-butenal was also tested in vivo for its ability to induce unscheduled DNA repair in rat liver cells at oral doses up to 700 mg/kg bw/day under OECD TG 486 and GLP conditions. There was no evidence that 3-methyl-2-butenal or its metabolites induced damage to DNA (BASF AG, 2001b).

Conclusion

In-vitro, 3-methyl-2-butenal revealed no mutagenic effect in the classical Ames test using with base pair substitution and frame shift tester strains. Positive effects with TA 100 with and without metabolic activation are ambiguous, since the results were not consistent between the experiments, the dose-relationship was not clear, and interference with cytotoxic effects could not be excluded. The results of the in vivo micronucleus test and the in vivo UDS assay clearly demonstrate the lack of any significant mutagenicity in vivo.

3.1.7 Carcinogenicity

No specific study concerning the investigation of a carcinogenic potential is available.

3.1.8 Toxicity for Reproduction

Effects on Fertility

Effects on reproduction and fertility were examined in a rat recently conducted one-generation study according to OECD TG 415 under GLP conditions where 3-methyl-2-butenal was administered to groups of 25 male and 25 female rats via drinking water at concentrations of 50, 200, and 800 ppm (see also chapter 3.1.5). This resulted in doses of ca. 6, 21, and 77 mg/kg bw/day for both sexes (BASF AG, 2002j). Administration of the test substance in the drinking water was chosen for the following reasons: Application in the diet was not possible due to stability problems and long-term administration by inhalation or by gavage was considered as critical due to the corrosive properties of the substance. Gross and histopathological examination of the sexual organs revealed no-substance-related adverse effects. Signs of distinctly impaired palatability of the drinking water solutions occurred in both genders of the F0 parents at 800 ppm and food consumption was also reduced in males. The clinical, gross and histopathological examinations yielded no indications for substance-induced systemic toxicity in the F0 parental animals up to and including 800 ppm. Reproductive performance and fertility were not affected at any dose level as evidenced by unchanged indices for male and female mating, fertility and litter size. Progeny development was equally unaffected as evidenced by unchanged birth and lactation live indices, unchanged sex ratio, and unchanged pup weight at delivery and pup body weight development weight until weaning.

Therefore, under the conditions of the study the NOAEL for reproductive performance and fertility, systemic toxicity and developmental toxicity was 800 ppm (about 77 mg/kg bw/day) for the F0 parental animals and their progeny. The NOEL for the parental animals was fixed at 200 ppm (about 21 mg/kg bw/day) due to reductions in water and/or food consumption at the top dose level in both gender.

Conclusion

Based on the results of a well conducted one-generation study (OECD TG 415) in rats with administration of 3-methyl-2-butenal in the drinking water the NOAEL for reproductive performance and fertility, systemic toxicity and developmental toxicity was found at the highest tested dose of 800 ppm (about 77 mg/kg bw/day) for the F0 parental animals and their progeny. No higher dose levels were achievable using drinking water due to palability problems that were already noted at the highest tested concentration.

Developmental Toxicity

3-Methyl-2-butenal was tested for its prenatal developmental toxicity in rats according to OECD TG 414 and under GLP conditions. Since in the initial study the high dose of 450 mg/kg bw/day turned out to be toxic (BASF AG, 2002k) a second study with 300 mg/kg bw/day was conducted (BASF AG, 2002l). Overall, dose levels of 50, 150, and 300 mg/kg bw/day were tested by gavage. The test substance was administered as an aqueous suspension to 25 time-mated female rats per group on day 6 through day 19 post coitum (p.c.). 25 control rats were dosed with the vehicle only (0.5 % Carboxymethylcellulose CB 30.000 in doubly distilled water). On day 20 p.c., all surviving females were sacrificed and assessed by gross pathology. For each dam, corpora lutea were counted and number and distribution of implantation sites were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external findings. Thereafter, nearly one

half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal findings (incl. cartilage).

The high and mid dose induced transient salivation in most animals at 300 mg/kg bw/day and in a few animals at 150 mg/kg bw/day shortly after treatment from day 6 p.c. onwards. This was very likely due to the intensive tasting properties of the test substance. Salivation was therefore assessed as a local effect without being adverse. The food consumption and the body weight development of the dams were not impaired at any dose and there were no adverse findings at necropsy.

Pre- and postimplantation loss was not changed at any dose, nor was the proportion of viable fetuses. Fetal body weight and sex distribution was unchanged. Fetal external and histopathological examination did not reveal any increase of malformation or variations compared to the concurrent or historical controls.

Therefore, 3-methyl-2-butenal was not developmental toxic and there were no signs of teratogenicity at doses up to and including 300 mg/kg bw/day. Higher dose levels could not be tested due to severe toxicity or mortality (5/25) in dams as noted at 450 mg/kg bw/day. Thus, 3-methyl-2-butenal provoked no signs of embryo- or fetotoxicity or teratogenicity at approx. the highest achievable dose level. The NOAEL for the mentioned endpoints was 300 mg/kg bw/day in these studies.

Conclusion

In a prenatal developmental toxicity study (OECD TG 414), 3-methyl-2-butenal had no effects on the gestational parameters and induced no signs of embryo- and fetotoxicity, in particular no indications for teratogenicity. The NOAEL for maternal toxicity and prenatal developmental toxicity was found at 300 mg/kg bw/day.

3.2 Initial Assessment for Human Health

No specific studies are available regarding toxicokinetics and metabolism of the substance.

3-Methyl-2-butenal is of moderate acute oral and acute inhalation toxicity and of low acute dermal toxicity.

LD50 rat (oral): 690 mg/kg bw; symptoms: labored breathing, stagger, tremors, apathy.

LC50 rat (inhalation): 3,700 mg/m³/4h (vapor); symptoms: lacrimation, nasal discharges, closed lids, labored breathing, dyspnoea, stagger, tremors, apathy.

LD50 rabbit (dermal): 3,400 mg/kg bw; symptoms: dyspnoea, stagger, abdominal position, apathy, local skin irritation.

Undiluted 3-methyl-2-butenal is corrosive to the skin and highly irritating to the eyes. Vapors of the material are irritating to the skin, the eyes, and the respiratory tract. In a well documented Guinea pig maximization test 3-methyl-2-butenal showed a skin sensitizing potential.

In a 28-day inhalation study (OECD TG 412) with rats, the NOAEC for systemic toxicity was at the highest tested concentration of 300 ppm (1,020 mg/m3, approx. 252 mg/kg bw/day). Symptoms of irritancy were seen in the respiratory tract at 100 ppm in terms of focal metaplasia of respiratory epithelium of the nasal cavity into squamous epithelium, changes in cartilage of larynx, and in some cases atrophy of the olfactory epithelium at 300 ppm. Purulent rhinitis in all males and some females was seen at 300 ppm. This was regarded to be responsible for reduced body weight development in both sexes (-59.8 % in males and -39.7 % in females compared to controls) and impaired terminal body weight in the high dose males (-14.1 %), rather than systemic toxicity.

There were no other findings which would indicate systemic toxicity. The NOAEC for local irritation was determined to be 30 ppm (100 mg/m3, approx. 25 mg/kg bw/day), the LOAEC was 100 ppm (340 mg/m3, approx 84 mg/kg bw/day) due to the histopathological changes observed in the respiratory tract.

As part of a one-generation study in rats, 3-methyl-2-butenal was administered subchronically at concentrations ranging from 50 - 800 ppm (about 6 – 77 mg/kg bw/day) via the drinking water for a period of 18 weeks. The highest test concentration (about 77 mg/kg bw/day) did not lead to any adverse findings in both sexes of the F0 parental animals. No higher dose levels were achievable using drinking water due to palability problems that were already noted at the highest tested concentration.

In vitro studies of the genotoxicity (Ames test) failed to produce consistent results. 3-methyl-2-butenal revealed no mutagenic effect in the classical Ames test whereas positive effects were reported from a Liquid Suspension Assay in the strain TA 100 with and without metabolic activation. In the mouse micronucleus test (OECD TG 474) the material was not clastogenic nor did it impair chromosome distribution during mitosis. No unscheduled DNA repair was induced in rat liver cells by the test material or metabolites in an in vivo test system according to OECD TG 486.

In a one-generation study (OECD TG 415) in rats with administration of 3-methyl-2-butenal in the drinking water, the NOAEL for reproductive performance and fertility, systemic toxicity and developmental toxicity was found at the highest tested dose of 800 ppm (about 77 mg/kg bw/day) for the F0 parental animals and their progeny.

In a prenatal toxicity study (OECD TG 414), 3-methyl-2-butenal had no effects on the gestational parameters and induced no signs of embryo- and fetotoxicity, in particular no indications for teratogenicity. The NOAEL for maternal toxicity and prenatal developmental toxicity was found at 300 mg/kg bw/day. A higher dose (450 mg/kg bw/day) could not be evaluated since it leads to severe toxicity or was lethal for the dams.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

The following acute toxicity tests with aquatic organisms are available:

Leuciscus idus	LC_{50} (96h) 17.6 mg/l	BASF AG, 1988b
Daphnia magna	EC_{50} (48h) 13.5 mg/l	BASF AG, 1988c
Scenedesmus subspicatus	E_rC_{50} (72h) 22.4 mg/l E_bC_{50} (72h) 16.7 mg/l	BASF AG, 1988d and 2002m

In addition, also effect values for microorganisms are available:

Tetrahymena pyriformis	EC_{50} (40h) 68 mg/l	Schultz and Cronin, 1999
Pseudomonas putida	EC_{50} (17h) 178 mg/l	BASF AG, 1990
Activated sludge	EC_{20} (0.5h) about 35 mg/l	BASF AG, 2002n

All tests were performed in static, open test systems. Since no substance specific concentration control analysis was performed, the effect values are related to the nominal concentrations. However, due to the vapor pressure of 3-methyl-2-butenal and its moderate volatility, an evaporation from the open test system was likely to have occurred.

Therefore, the volatility was determined by measuring the remaining test substance using TOC measurements under comparable test conditions but without the corresponding test organisms (BASF AG, 2003b). Two parallels for each test system using 100 mg/l 3-methyl-2-butenal were run. The values obtained after the corresponding test periods are as follows (Table 2):

Table 2: Evaporation of 100 mg/l 3-methyl-2-butenal in the different test systems

Species	**Test period**					**Geometric mean**
	0h	24h	48h	72h	96h	
L. idus	100 %	77 %	75 %	64 %	54 %	72 %
D. magna	100 %	88 %	80 %	/	/	89 %
S. subspicatus	100 %	94 %	85 %	78 %	/	89 %

In the approaches in open test systems for daphnids and algae the concentration of 3-methyl-2-butenal, on the basis of TOC measurements, dropped from the beginning until the end to approximately 80 %, whereas in the approach for the fish only 54 % of the tests compound could be found after 96 h.

Using TOC measurements and the derived geometric means of all measured values between the start and the end of the treatments in the corresponding tests systems for algae and daphnids recovery rates of approximately 89 % could be calculated. Thus, the volatility of the test compound from these systems (daphnia and algae) is below 20 % and can therefore be neglected (BASF AG, 2003c).

For the acute test with the fish *L. idus* based on the nominal LC_{50} of 17.6 mg/l an effective LC_{50} (96h) of 12.7 mg/l can be estimated in considerations of a recovery rate of 72 % (geometric mean). Based on these data, 3-methyl-2-butenal is considered as hazardous to aquatic organisms.

Results from prolonged or chronic studies are not available.

4.2 Terrestrial Effects

There are no data available concerning the toxicity to soil dwelling organisms, terrestrial plants or other non-mammalian terrestrial organisms.

4.3 Other Environmental Effects

There are no data available.

4.4 Initial Assessment for the Environment

3-Methyl-2-butenal is a colorless-faint yellowish liquid with a water solubility of 110 g/l at 20 °C. The calculated Henry´s Law Constants are 0.57 $Pa*m^3$/mole and 2.85 $Pa*m^3$/mole. Due to the

measured log K_{ow} of 0.53 and the calculated log K_{oc} of 0.9 (K_{oc} = 7.9), bio- and geoaccumulation are not to be expected. Distribution modeling using Mackay Level I indicates water and air to be the main target compartments. 3-Methyl-2-butenal was shown to be readily biodegradable according to OECD criteria in a CO_2-Headspace test with 80 - 90 % degradation within 28 days (criteria of a 10-days time window fulfilled). Hydrolysis at environmental pH conditions is not expected according to the structure. In the atmosphere, the substance will be indirectly photodegraded by reaction with OH-radicals (calculated $t_{½}$ = 8.2 h) or ozone (calculated $t_{½}$ = 23.2 h).

The following aquatic effect concentrations are available from tests preformed in open systems. Only for the fish toxicity the value was corrected due to losses of the test compound by its volatility:

Leuciscus idus LC_{50} (96h) 17.6 mg/l (nominal) and the corrected LC_{50} (96h) 12.7 mg/l (effective); *Daphnia magna* EC_{50} (48h) 13.5 mg/l; *Scenedesmus subspicatus* ErC_{50} (72h) 22.4 mg/l, EbC_{50} (72 h) 16.7 mg/l.

Prolonged or chronic studies are not available.

Based on the most sensitive species *L. idus* with a LC_{50} (96h) of 12.7 mg/l (recalculated due to moderate volatility), a $PNEC_{aqua}$ of approximately 13 µg/l can be derived by applying an assessment factor of 1,000, according to the Technical Guidance Document for the EU risk assessment procedure.

5 RECOMMENDATIONS

Environment: The chemical is currently of low priority for further work. 3-Methyl-2-butenal possesses properties indicating a hazard for the environment. Although these hazards do not warrant further work as they are related to acute toxicity which may become evident only at very high exposure levels, they should nevertheless be noted by chemical safety professionals and users.

Human Health: The chemical is currently of low priority for further work. 3-Methy-2-butenal possesses corrosive and sensitizing properties indicating a hazard for human health. Given the main use as a chemical intermediate in closed systems and the very low content of the substance in consumer products in the Sponsor country, the substance is considered to be of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

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ANNEX

Details of the literature search used

The data banks searched are indicated below.

Toxicology

Date of last literature search: 17 October 2002

JETOC

RTECS

AGRICOLA

CABA

CANCERLIT

TOXCENTER

TOXLINE

JICST-EPLUS

LIFESCI

TOXLIT

EMBASE

ESBIOBASE

EMBAL

HEALSAFE

CSNB

MEDLINE

IRIS

ATSDR TOX. PROFILES

atsdr TOX: FAQS

chemfinder

civs

gestis

ginc

nicnas

ntp

Ecology

Date of last literature search: 17 October 2002

AQUASCI

BIOSIS

EMBASE

ESBIOBASE.

LIFESCI

OCEAN

POLLUAB

SCISEARCH

TOXCENTER

TOXLINE

ULIDAT

datalog

chemfate

biodeg

aquire

HSDB

I U C L I D

D a t a S e t

Existing Chemical ID: 107-86-8
CAS No. 107-86-8
EINECS Name 3-methyl-2-butenal
EC No. 203-527-6
Molecular Weight 84.12
Molecular Formula C5 H8 O

Producer Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Substance Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Memo: master

Printing date: 08-MAR-2004
Revision date:
Date of last Update: **05-MAR-2004**

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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, SIDS

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Flag: Critical study for SIDS endpoint
04-NOV-2002

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Country: Japan
Phone: 81-3-3277-6683
Telefax: 81-3-3277-6718

Flag: Critical study for SIDS endpoint
12-NOV-2002

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

Mol. Formula: C5 H8 O
Mol. Weight: 84.12 g/mol

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002

1.1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: >= 96 - % w/w
Colour: colourless-faint yellowish
Odour: pungent

Method: CGC (Capillary Gas Chromatography)
Flag: non confidential, Critical study for SIDS endpoint
28-MAY-2003 (1) (2)

Purity type: typical for marketed substance
Purity: 98.5 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

1.1.2 Spectra

1.2 Synonyms and Tradenames

.beta.,.beta.-Dimethylacrolein

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

.beta.,.beta.-Dimethylacrylic aldehyde

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

.beta.-Methylcrotonaldehyde

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

2-Butenal, 3-methyl- (9CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

3,3-Dimethylacrolein

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

3-Methyl-2-butenal

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

3-methylbuten-2-al-1

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

3-Methylcrotonaldehyde

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Crotonaldehyde, 3-methyl- (7CI, 8CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Prenal

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Senecialdehyde (6CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Senecioaldehyde

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

1.3 Impurities

CAS-No: 556-82-1
EC-No: 209-141-4
EINECS-Name: 3-methylbut-2-en-1-ol
Mol. Formula: C5 H10 O
Contents: <= 1 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
22-APR-2003 (4)

CAS-No: 763-32-6
EC-No: 212-110-8
EINECS-Name: 3-methylbut-3-en-1-ol
Mol. Formula: C5 H10 O
Contents: <= 1 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
22-APR-2003 (4)

EINECS-Name: 3-methyl-3-buten-al
Mol. Formula: C5 H8 O
Contents: <= 1 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (4) (3)

EINECS-Name: 3-Methyl-3-butanol
Contents: <= .3 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

EINECS-Name: 3-Methyl formate
Contents: <= .1 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

CAS-No: 763-32-6
EC-No: 212-110-8
EINECS-Name: 3-methylbut-3-en-1-ol
Mol. Formula: C5 H10 O
Contents: <= .1 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

CAS-No: 50-00-0
EC-No: 200-001-8
EINECS-Name: formaldehyde
Mol. Formula: C H2 O
Contents: <= .02 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

1.4 Additives

1.5 Total Quantity

Remark: Production volumes for the year 2001:

USA	: 0 t
Europe (incl. Germany)	: 5,000 - 10,000 t
Asia	: 1,000 - 3,000 t
World	: 6,000 - 13,000 t

Flag: Critical study for SIDS endpoint
24-JUL-2003

1.6.1 Labelling

Labelling: provisionally by manufacturer/importer
Symbols: (C) corrosive
R-Phrases: (10) Flammable
(34) Causes burns
(20/22) Harmful by inhalation and if swallowed
(43) May cause sensitization by skin contact
S-Phrases: (36/37/39) Wear suitable protective clothing, gloves and eye/face protection
(26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

1.6.2 Classification

Classified: provisionally by manufacturer/importer
Class of danger: corrosive
R-Phrases: (34) Causes burns

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Classified: provisionally by manufacturer/importer
Class of danger: flammable
R-Phrases: (10) Flammable

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Classified: provisionally by manufacturer/importer
Class of danger: harmful
R-Phrases: (20/22) Harmful by inhalation and if swallowed

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Classified: provisionally by manufacturer/importer
Class of danger: irritating
R-Phrases: (43) May cause sensitization by skin contact

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Non dispersive use

Flag: non confidential, Critical study for SIDS endpoint
15-DEC-1993

Type: type
Category: Wide dispersive use

Remark: Estimated volumes are very low (< 10 t/a) for use as reconstitution of essential oils and other natural products.
Flag: non confidential, Critical study for SIDS endpoint
22-JUL-2003

Type: industrial
Category: Chemical industry: used in synthesis

Remark: Synthesis of fine chemicals (> 90 % of the world production is used for synthesis of citral)
Flag: non confidential, Critical study for SIDS endpoint
22-JUL-2003

Type: use
Category: Intermediates

Flag: non confidential, Critical study for SIDS endpoint
15-DEC-1993

1.7.1 Detailed Use Pattern

12-NOV-2002

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: 2-Alkenals are obtained by the reaction of unsaturated alkyl halides with the sodium salts of secondary nitrohydrocarbons.

(CH3)2C=CH-(CH2)2-C(CH3)=CH-CH2-X + R2C=N+(ONa)O- ->
(CH3)2C=CH-(CH2)2-C(CH3)=CH-CHO + R2C=NOH + NaX

Another possibility is the treatment of acetals with vinyl ethers in the presence of boron trifluoride to give the corresponding beta-alkoxyacetals. Under the influence of acids these are converted into alpha,beta-unsaturated aldehydes.

CH3-CH2-O-CHR-O-CH2-CH3 + CH2=CH-O-CH2-CH3 -(BF3)->
(CH3-CH2-O-)2CH-CH2-CHR-O-CH2-CH3 -(H3O+)-> R-CH=CH-CHO

Flag: non confidential, Critical study for SIDS endpoint
10-JUL-2003 (5)

Orig. of Subst.: Synthesis
Type: Production

Remark: At BASF AG 3-Methyl-2-butenal is made from isobutene and formaldehyde followed by oxidative dehydrogenation of the methylbutenol.
(Patent DE 3612213; BASF AG, 1996)
Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (3)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value: other: not listed in the MAK- and BAT-value list.

Flag: non confidential, Critical study for SIDS endpoint
08-JUL-2003 (6)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany), Annex 2
Labelled by: other: VwVwS (Germany), Annex 2
Class of danger: 2 (water polluting)

Remark: ID-Number: 1145
Flag: non confidential, Critical study for SIDS endpoint
22-APR-2003 (7)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

02-DEC-1992

1.8.6 Listings e.g. Chemical Inventories

Type: other: EPA
Additional Info: EPA No. P 87-762

Remark: EPA FLAGS:
P Commenced PMN
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: EINECS
Additional Info: EINECS No. 203-527-6

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: ENCS
Additional Info: ENCS No. 2-2461

Remark: ENCS CLASSIFICATION:
Low Molecular Chain-like Organic Compounds.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: DSL

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (8)

1.9.1 Degradation/Transformation Products

CAS-No: 630-08-0
EC-No: 211-128-3
EINECS-Name: carbon monoxide

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

CAS-No: 1333-74-0
EC-No: 215-605-7
EINECS-Name: hydrogen

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

CAS-No: 115-11-7
EC-No: 204-066-3
EINECS-Name: 2-methylpropene

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

1.9.2 Components

1.10 Source of Exposure

1.11 Additional Remarks

Memo: German "Flammable Liquids" classification (VbF): AII

Flag: non confidential, Critical study for SIDS endpoint
05-NOV-2002 (1)

Memo: Hazardous reactions:
Risk of self-ignition when a large surface area is produced due to fine dispersion.

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (1)

Memo: Substances to avoid:
acids and alkalies

Flag: non confidential, Critical study for SIDS endpoint
02-JUN-2003 (1)

1.12 Last Literature Search

Type of Search: Internal and External
Chapters covered: 5.10
Date of Search: 06-NOV-2002

06-FEB-2003

Chapters covered: 1
Date of Search: 28-MAY-2003

Flag: non confidential, Critical study for SIDS endpoint
28-MAY-2003

Type of Search: Internal and External
Chapters covered: 3, 4
Date of Search: 15-OCT-2002

Flag: non confidential, Critical study for SIDS endpoint
08-JUL-2003

Type of Search: Internal and External
Chapters covered: 2
Date of Search: 15-OCT-2002

Flag: non confidential, Critical study for SIDS endpoint
08-JUL-2003

Type of Search: Internal and External
Chapters covered: 5

Date of Search: 15-OCT-2002

Flag: non confidential, Critical study for SIDS endpoint

08-JUL-2003

1.13 Reviews

Memo: BUA-report 194

Flag: non confidential, Critical study for SIDS endpoint
28-MAY-2003

2.1 Melting Point

Value: -76.8 degree C

Method: other: calculated via MPBPWIN v1.40
Year: 2002

Remark: Mean melting point according to Joback, Gold, Ogle methods
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
24-JUL-2003 (9)

Value: < -20 degree C

Method: other: no data
GLP: no
Test substance: no data

Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
12-JUN-2003 (1)

2.2 Boiling Point

Value: = 133 degree C

Reliability: (4) not assignable
secondary literature
03-FEB-2004 (10)

Value: = 134.5 degree C at 1013.4 hPa

Method: other: determination of vapour pressure
Year: 1979
GLP: no

Test substance: 3-methyl-2-butenal, purity >98.2 weight% (GC)
Reliability: (2) valid with restrictions
Test method scientifically acceptable, data conclusive
01-JUL-2003 (11)

Value: = 135.9 degree C at 1013.3 hPa

Method: other: dynamic under nitrogen atmosphere
Year: 1985
GLP: no

Remark: The boiling point value was calculated based on measured vapor pressure at 0.04 to 135.51°C (see also chapter 2.4 for details).
Test substance: 3-methyl-2-butenal, purity 95.23 % (GC)
Reliability: (2) valid with restrictions
Test method scientifically acceptable, data conclusive
Flag: Critical study for SIDS endpoint
01-JUL-2003 (12)

Value: = 136 degree C at 1013 hPa

Reliability: (4) not assignable
Manufacturer/producer data without proof
09-MAY-2000 (1)

2.3 Density

Type: density
Value: = .8722 at 20 degree C

Reliability: (4) not assignable
secondary literature
03-FEB-2004 (10)

Type: density
Value: = .876 g/cm³ at 20 degree C

GLP: no
Test substance: no data

Reliability: (4) not assignable
Secondary quotation of measured data. Original test report no longer available.
Flag: Critical study for SIDS endpoint
05-DEC-2002 (13)

Type: density
Value: = .88 g/cm³ at 20 degree C

Reliability: (4) not assignable
Manufacturer/producer data without proof
04-DEC-2002 (1)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = 7.5 hPa at 20 degree C

Result: 40 hPa at 50°C
Reliability: (4) not assignable
Manufacturer/producer data without proof
09-MAY-2000 (1)

Value: = 7.5 hPa at 20 degree C

Method: other (measured): dynamic with nitrogen
Year: 1985
GLP: no
Test substance: other TS

Method: dynamic with nitrogen

Result: temperature (°C) vapour pressure (hPa)

0.04	1.890
9.92	3.870
19.96	7.460
36.82	19.990
61.69	69.990
69.72	99.850
97.67	299.860
123.39	699.800
129.91	849.700
135.51	999.800

Further data derived from the Vp-curve:

20	7.5
50	40
100	approx. 320

Test substance: 3-methyl-2-butenal, purity ca. 95%
Reliability: (2) valid with restrictions
Study well documented, data conclusive
Flag: Critical study for SIDS endpoint
04-DEC-2002 (12)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = .53 at 25 degree C

Method: other (measured): comparable to OECD 107
Year: 1988
GLP: no

Remark: mean value of three measurements
Test substance: 3-methyl-2-butenal, purity 97.4 % (GC)
Reliability: (2) valid with restrictions
Test method comparable to guideline study
Flag: Critical study for SIDS endpoint
05-DEC-2002 (14)

Partition Coeff.: octanol-water
log Pow: = 1.15

Method: other (calculated): KOWWIN v1.66
GLP: no

Reliability: (2) valid with restrictions
Accepted calculation method
05-DEC-2002 (15)

2.6.1 Solubility in different media

Solubility in: Water
Value: ca. 110 g/l at 15 degree C

Reliability: (4) not assignable

Manufacturer/producer data without proof
04-DEC-2002 (1)

Solubility in: Water
Value: = 110 g/l at 20 degree C

Method: other: see below
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: 1. potentiometric determination of the TS (BASF, 1973; no information about the TS is given in the report)
2. GC analytics (BASF, 1979)
Remark: The unit is expressed as 11 wt% at 20°C resulting in a solubility of 110 g/l.
Result: water solubility:

= 11.0 weight% = 110 g/l at 20.0 °C
= 9.3 weight% = 93 g/l at 40.0 °C
= 8.7 weight% = 87 g/l at 65.0 °C
= 9.7 weight% = 97 g/l at 94.8 °C
(BASF, 1973)

= 10.1 weight% = 101 g/l at 32.3 °C
= 9.78 weight% = 97.8 g/l at 32.3 °C
= 8.66 weight% = 86.6 g/l at 47.2 °C
= 9.90 weight% = 99 g/l at 94.4 °C
(BASF, 1979)

Test substance: 3-methyl-2-butenal, purity >98.2 weight% (GC) (BASF, 1979)
Reliability: (2) valid with restrictions
Test method scientifically acceptable, data conclusive
Flag: Critical study for SIDS endpoint
26-JAN-2004 (16) (11)

2.6.2 Surface Tension

Test type: other: not specified
Value: = 28 mN/m at 0 degree C

Method: other: not specified
GLP: no
Test substance: no data

Remark: = 24 mN/m at 40 °C
= 20 mN/m at 80 °C
Test substance: The result refers to a neat liquid.
Reliability: (4) not assignable
Secondary quotation of measured data. Original test report no longer available.
Flag: Critical study for SIDS endpoint
05-DEC-2002 (17)

2.7 Flash Point

Value: = 37 degree C

Method: other: DIN 51 755
GLP: no
Test substance: no data

Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
12-JUN-2003 (1)

2.8 Auto Flammability

Value: = 145 degree C

Method: other: DIN 51 794
GLP: no
Test substance: no data

Remark: The value corresponds to the ignition temperature.
Hazardous reactions: Risk of self-ignition when a large surface area is produced due to fine dispersion.
Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
12-JUN-2003 (1)

2.9 Flammability

Result: flammable

Test substance: no data

Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
12-JUN-2003 (1)

2.10 Explosive Properties

Result: not explosive

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: The substance has no chemical groups indicating explosive properties, because the chemical structure does not contain fluorine or chlorine and the compound contains oxygen chemically bonded only to carbon.
This statement agrees with the recommendations on the transport of Dangerous Goods, Manual of Tests and Criteria, Appendix 6 (third revised edition) of the United Nations.
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
21-FEB-2002 (18)

2.11 Oxidizing Properties

Result: no oxidizing properties

GLP: no

Test substance: as prescribed by 1.1 - 1.4

Remark: The substance has no oxidizing properties, because the chemical structure does not contain fluorine or chlorine and the compound contains oxygen chemically bonded only to carbon. This statement agrees with the recommendations on the transport of Dangerous Goods, Manual of Tests and Criteria, Appendix 6 (third revised edition) of the United Nations.
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
19-FEB-2002 (18)

2.12 Dissociation Constant

2.13 Viscosity

Test type: other: not specified
Value: = .98 mPa s (dynamic) at 0 degree C

Method: other: no data
GLP: no
Test substance: no data

Result: Further data presented:

Temperature[°C]	dyn. Viscosity[mPa x s]
0	0.98
60	0.46
120	0.28

Reliability: (4) not assignable
Secondary quotation of measured data. Original test report no longer available.
06-DEC-2002 (17)

Test type: other: not specified
Value: = 1 mPa s (dynamic) at 20 degree C

Method: other: no data
GLP: no
Test substance: no data

Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
08-JUL-2003 (1)

2.14 Additional Remarks

Memo: Other safety aspects

Remark: Explosion limits: 1.8 - 7 vol.-%
Reliability: (4) not assignable
Manufacturer/producer data without proof

04-DEC-2002 (1)

Memo: Refraction index

Remark: The refraction index is 1.4526.
Reliability: (4) not assignable
secondary literature
03-FEB-2004 (10)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: 700000000000 molecule/cm³
Rate constant: = .00000000000000001183 cm³/(molecule * sec)
Degradation: = 50 % after 23.2 hour(s)

Method: other (calculated): AOP (v1.90)
Year: 2002

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
30-JUN-2003 (19)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1500000 molecule/cm³
Rate constant: = .000000000046696 cm³/(molecule * sec)
Degradation: = 50 % after 2.8 hour(s)

Method: other (calculated): AOP (v1.90)
Year: 2002

Remark: Calculation of t1/2 based on 12 hour-day.
Reliability: (2) valid with restrictions
Accepted calculation method
01-JUL-2003 (19)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .000000000046696 cm³/(molecule * sec)
Degradation: = 50 % after 8.2 hour(s)

Method: other (calculated): AOP (v1.90)
Year: 2002

Remark: Calculation of t1/2 based on 24 hour-day.
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
01-JUL-2003 (19)

3.1.2 Stability in Water

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

Type of measurement: other: concentration in oil of root material via GC/MS
Medium: other: wild ginger

Remark: Less than 0.05 % of the steam-distilled oil obtained from

root material of wild ginger (Asarum canadense) [total oil yield 3 %] composed of 3-methyl-2-butenal (Analysis by GC-MS).

Reliability: (2) valid with restrictions
scientifically acceptable, well documented and comprehensible
08-JUL-2003 (20)

Medium: food

Remark: One cultivated (Holland) and two wild varieties of blackberries (Spain and Burgundy) were collected and stored at -20°C. Two extracts of each were performed using dichloromethane and 50 % saccharose were added to one of them.

0.04 % and 0.2 % (using 50 % sugar) were found in wild variety from Burgundy. In the wild variety from Spain 0.24 and 0.34 % (with 50 % sugar) were found, whereas in the cultivated sort no 3-methyl-2-butenal could be detected. Adding sugar did not have an significant effect on the occurence of the substance.

Reliability: (2) valid with restrictions
scientifically acceptable, well documented and comprehensible
08-JUL-2003 (21)

Medium: food

Remark: 0.36 % of the condensable lipid fraction of raw beef composed of 3-methyl-2-butenal after supercritical CO2-extraction, while this compound was not identified in the volatile fraction of the extract.

Reliability: (2) valid with restrictions
scientifically acceptable, well documented and comprehensible
08-JUL-2003 (22)

Medium: food

Remark: 9 ug/kg 3-methyl-2-butenal were detected in an extract from dripping fat of roasting chicken after vacuum distillation followed by analysis via GC/FID.

Reliability: (2) valid with restrictions
scientifically acceptable, well documented and comprehensible
08-JUL-2003 (23)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: calculated

Remark: Based on the distribution model using the regression equation, log Koc = 0.544 logPow + 1.377 (Kenaga and Goring,1980), and the oil-water partition coefficient with log Pow = 0.53 (measured) [see also 2.5], the soil adsorption coefficient results in Koc of 46 (logKoc = 1.66).

Based on the distribution model using the regression equation, log Koc = -0.55 logCs + 3.64 (Kenaga and Goring,1980), and the water solubility Cs of 110,000 ppm [see

also 2.6.1], the soil adsorption coefficient results in Koc of 7.4 (logKoc = 0.86).

This indicates very high mobility of the TS in soil.

Reliability: (2) valid with restrictions
Accepted calculation method

09-JUL-2003 (24)

Type: adsorption
Media: water - soil
Method: other: calculated via PCKOCWIN v1.66
Year: 2002

Result: Based on the calculation programme PCKOCWIN v1.66, the soil adsorption coefficient Koc is 7.878 (log Koc: 0.8964). This indicates high mobility in soil.

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint

30-JUN-2003 (25)

Type: volatility
Media: water - air
Method: other: calculated

Result: The calculation by using the vapour pressure (Ps = 750 Pa) and the water solubility (Cs = 1307.6 mol/m3) (as cited by Thomas, 1982) results in a Henry´s Law-Constant of 0.57 Pa*m3/mol at 20°C.
This indicates moderate volatility of 3-methyl-2-butenal from the hydrosphere.

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint

09-JUL-2003 (26)

Type: volatility
Media: water - air
Method: other: calculated via HENRYWIN v3.10

Result: Henry's Law Constants based on the model calculation are:

Bond Est : 8.91 Pa*m3/mole
Group Est: 2.85 Pa*m3/mole

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint

26-JAN-2004 (27)

Type: other: volatilization from surface water
Media: water - air
Method: other: EPIWIN v3.10

Result: 3-methyl-2-butenal in surface water will be subject to slowly volatilization. Using an estimated Henry´s law constant of 2.85 Pa*m³/mol (Group estimate), a half-life for volatilization of the chemical from a 1 m deep river with a current velocity of 1 m/sec and a wind velocity of 5 m/sec has been estimated to be 20.1 hours. A volatilization half-life

from a 1 m deep lake with a current velocity of 0.05 m/sec and a wind velocity of 0.5 m/sec has been calculated to be 295.6 hours.

Reliability: (2) valid with restrictions
Accepted calculation method

08-JUL-2003 (28)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: other (calculation): via Mackay Level I V2.11
Year: 2002

Remark: The following physical and chemical parameter were used for the calculation:
molecular mass = 84.12 g/mol
data temperature = 20°C
logKow = 0.53
water solubility = 110000 g/cm³
vapour pressure = 750 Pa
melting point = -76.9°C
(the Henry's Law Constant calculated by the programm itself was: 0.57 Pa*m³/mol)

	Volume (m³)	Density (kg/m³)	org. C (g/g)	fish lipid (g/g)
Air	6.0E+09	1.185		
Water	7.0E+06	1000		
Soil	45000	1500	0.02	
Sediment	21000	1300	0.05	
susp. Sed.	35	1500	0.167	
Fish	7	1000		0.05
Aerosole	0.012	1500		

Result: 3-methyl-2-butenal will be mainly distributed into the compartments water and air.
air: 16.5 %
water: 83.5 %
soil: 0.02 %
sediment: 0.02 %
susp.sed.: 0.0001 %
fish: 1.41*E-05 %
aerosol: 2.65*E-06 %

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint

08-JUL-2003 (29)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic, non-adapted
Concentration: 20 mg/l related to DOC (Dissolved Organic Carbon)
28 mg/l related to Test substance
Degradation: 80 - 90 % after 28 day(s)
Result: readily biodegradable
Kinetic: 3 day(s) = 29 %

7 day(s) = 62 %
10 day(s) = 75 %
21 day(s) = 77 %
28 day(s) = 82 %

Control Subst.: Aniline
Kinetic:
3 day(s) = 55 %
7 day(s) = 81 %

Method: other: ISO 14593: CO2-Headspace Test
Year: 1999
GLP: yes

Remark: In the CO2-Headspace Test the biodegradation of a substance can be determinmed by measuring the CO2 evolution.

Within this test 3 replicates for the test substance and the reference substance as well as for the inhibiton control and the examination of the physical and chemical (abiotic) elimination were used.

As inoculum 4 mg/l dry substance (activated sludge from a laboratory wastewater treatment plant treating municipal sewage) was used.

As reference control the test substance aniline using a concentration of 20 mg/L (nominal) was used.

The biodegradation was calculated using the degree of the relation of TIC (total inorganic carbon) to ThIC (theoretical inorganic carbon [% TIC/ThIC].

All validity criteria were fulfilled.

Result: After 1 day 3-methyl-2-butenal was degraded up to 10 %. After 11 days a biodegradation of > 75 % could be observed.
The reference substance aniline was > 60 % degraded within 14 days.
The physical-chemical (abiotic) elimination of the test substance at the end of the test was 1 %.
The inhibition control showed the following kinetic:
3 day(s) = 46 %
7 day(s) = 70 %
10 day(s) = 79 %
14 day(s) = 85 %
28 day(s) = 89 %

Test substance: 3-methyl-2-butenal; purity: 97.9 %
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
10-JUL-2003 (30)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 400 mg/l related to DOC (Dissolved Organic Carbon)
678 mg/l related to Test substance
Contact time: 10 day(s)
Degradation: = 99 % after 10 day(s)
Result: other: easily eliminable from water, inherently biodegradable

Method: Directive 88/302/EEC, C.9
Year: 1987

GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: lag-Phase: about 3 d
Test condition: Inoculum: 1 g/l (dry matter)[activated sludge from BASF WWTP]
pH: 6,0 - 8.2
Reliability: (2) valid with restrictions
guideline study, basic data given
08-JUL-2003 (31)

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: other: no data
Year: 1987
GLP: no
BOD5: = 1365 mg/l

C O D

Method: other: no data
Year:
GLP: no
COD: = 2370 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = .58
Method:
Result: COD = 2370 mg/g
BOD5 = 1365 mg/g

BOD5/COD = 0.58
Reliability: (2) valid with restrictions
Manufacturer/producer data without proof: data conclusive
Flag: Critical study for SIDS endpoint
26-JUN-2003 (31)

3.7 Bioaccumulation

BCF: = .56

Method: other

Method: The BCF is derived from log Kow using the following equation:
log BCF = 0.85*log Kow - 0.70
as cited in the TGD (May 2002).

For this calculation, the measured log Kow of 0.53 was used.
Result: logBCF = -0.2495
Accepted calculation method
Flag: Critical study for SIDS endpoint
01-JUL-2003 (32)

BCF: 1.53

Method: other: calculated, SRC - BCFWIN v2.14
Year: 2002
Test substance: as prescribed by 1.1 - 1.4

Result: log BCF = 0.184
Test condition: The EPIWIN calculation of BCF is derived from log Kow using the following formula:
log BCF = 0.77 log Kow - 0.70 + Correction factor

For this calculation, a log Kow of 1.15 and no correction factor was used.
Reliability: (2) valid with restrictions
Accepted calculation method
30-JUN-2003 (33)

3.8 Additional Remarks

Memo: Microbiological studies: Biodegradation and biotransformation

Remark: Several cultures of bacteria were isolated from soil under Austrian pines, among them Pseudomonas putida, Arthrobacter and Bacillus species able to utilize 3-methyl-2-buten-1-ol, a volatile alcohol produced in pine needles as sole carbon source.

These microorganism possess inducible dehydrogenases resulting in 3-methyl-2-buten-1-al which undergoes oxidation by a further dehydrogenase indicative of catabolism via 3-methylcrotonic acid.
Reliability: (2) valid with restrictions
Microbiological studies, based on accepted scientific principles.
01-JUL-2003 (34)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 10
LC0: = 10
LC50: = 17.6 calculated
LC100: <= 46
LC50 (effective, after evaporation) :
ca. 12.6

Method: other: according to DIN 38412, part 15
Year: 1982
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: The LC50 was calculated according to the ToxRat calculation program using a probit transformation. The resulting LC50 was 17.6 mg/L (nominal; confidence limit: 14.6 - 21.3 mg/L).

In an experiment for testing the volatility in an open system after 96h approximately 28 % (geometric mean of all measured TOC values between 0h and 96h) evaporated. Therefore, the LC50 can be corrected to be 12.7 mg/L (effective) after 96h.

Animals exposed to 21.5 mg/l (nominal) showed apathy (narcotic-like state) after 4 h. At 10 mg/l (nominal), no such effects were reported.

Test condition: The method used closely followed the guideline of DIN 38 412 ["Testverfahren mit Wasserorganismen (Gruppe L). Allgemeine Hinweise zur Planung, Durchführung und Auswertung biologischer Testverfahren (L1) und Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische - Fischtest (L15)"], June 1982, using a static procedure. One criteria of this guideline is that the corpulence factor K should be between 0.8 and 1.1 g/cm³ (equation for calculation: $K = 100*W/L^3$; W = weight in g; L = length in cm).

Fish: golden orfe (Leuciscus idus L., golden variety)
Body length: 6.9 - 7.9 cm
Body weight: 2.8 - 4.6 g
Corpulence factor K: 0.87 g/cm³
Number if fish per treatment: 10
Fish loading: 3.6 g/l
Test temperature: 21 - 23 °C
Photoperiod: 16 h light and 8 h dark
Water total hardness: approx. 2.5 mmol/l
Acid capacity: approx. 0.8 mmol/l
measured pH values:

concentration	pH				
(nominal, mg/l)	1h	24 h	48 h	72 h	96 h
10.0	7.5	7.5	7.4	7.4	7.6
21.5	7.5	7.4	7.4	7.5	7.5
46.4	7.4				
100.0	7.5				
control	7.8	7.7	7.7	7.5	7.5

measured oxygen concentrations
concentration
(nominal, mg/l): O2

	1h	24 h	48 h	72 h	96 h
10.0	6.5	6.5	6.0	6.5	7.4
21.5	6.2	6.7	7.0	7.4	7.4
46.4	7.0				
100.0	7.6				
control	6.2	7.3	7.4	7.3	7.3

Test concentrations: 10, 21.5, 46.4, and 100 mg/l following a range finding study, with respective volumes taken from an aqueous stock solution of 10 g/l.

The test substance was added to the test water without any prior treatment. Subsequently, the fish were added to the water.

Results refer to nominal concentrations.

Test substance: purity 98 %
Reliability: (2) valid with restrictions
Comparable to guideline study, in accordance with accepted national standards
Flag: Critical study for SIDS endpoint
05-MAR-2004 (35) (36) (37)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 6.25
EC50: = 13.5
EC100: = 25
EC50 (95-% Conf.) :
= 10.1 - 16.9

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year: 1984
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Results after 24 h were as follows:
EC0 = 25 mg/l
EC50 = 33.1 mg/l (95%-Conf. = 28.9 - 37.3 mg/l)
EC100 = 50 mg/l

In an experiment for testing the volatility in a open system after 48h only 11% (geometric mean of all measured TOC values between 0h and 48 h) evaporated. Therefore, no correction of the derived EC50 values was undertaken.
Test condition: Test water: pH value: 7.7 - 8.3
Total hardness: 2.7 mmol/l
Alkalinity up to pH 4.3: 0.80 mmol/l
Conductivity: 550 - 650 µS/cm
Illumination: artificial light (day:night-rhythm = 16h:8h)
Light intensity: 5 µE at a wave of 400 - 750 nm

Test temperature: 19 - 21 °C
Test volume: 10 ml
Volume/animals: 2 ml
Number of animals/vessel: 5
Total number of animals/concentration: 20

Check of the study: visually after 0, 3, 6, 24, and 48 h.

Nominal test concentrations: 3.125, 6.25, 12.5, 25, 50, 100 mg/l.

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measured pH values:

concentration (nominal, mg/l)	pH 0h	pH 48 h
3.125	7.99	7.89
6.25	7.99	7.85
12.5	8.00	7.75
25.0	7.99	7.58
50.0	7.99	7.53
100.0	7.97	7.54
control	8.04	7.93

measured oxygen concentrations

concentration (nominal, mg/l):	O2 0h	O2 48 h
3.125	8.37	8.37
6.25	8.33	8.17
12.5	8.23	7.92
25.0	8.26	7.51
50.0	8.98	7.37
100.0	9.06	7.28
control	8.41	8.59

Test substance: Purity >98 %
Reliability: (1) valid without restriction
Test method comparable to guideline study, in accordance with accepted national standards
Flag: Critical study for SIDS endpoint
24-JUL-2003 (38) (36)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 14.1
EC50: = 22.4
EC 90 : = 41.3

Method: other: Scenedesmus cell multiplication Inhibition Assay according to DIN 38412 Part 9 (Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Gruenalgen)
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Effect values (endpoints: growth rate and biomass) were recalculated according to OECD 201 guideline using linear regression analysis considering fluorescence values

mentioned in the report (BASF AG, Department of Ecology, unpublished data, 01/87/1059, 15.03.88), yielding 72 h growth rate values of ErC10 = 14.1 mg/L, ErC50 22.4 mg/l, ErC90 41.3 mg/l, and biomass values of EbC10 = 9.37 mg/L, EbC50 = 16.7 mg/L and EbC90 29.1 mg/L.

The algal cell densities in the control for the test duration of 72 h are as follow:
0 hours 55
24 hours 170 (+/- 12 StD)
48 hours 562 (+/- 37 StD)
72 hours 1426 (+/- 159 StD)

Original effect values as given in the report after 72 h are as follows:

EC20 = 14 mg/l
EC50 = 20 mg/l
EC90 = 30 mg/l

Original effect values as given in the report after 96 h are as follows:

EC20 = 17 mg/l
EC50 = 23 mg/l
EC90 = 30 mg/l

The toxicity after 96h is lower compared to the EC50 after 72h, and therefore the volatility of the test substance was determined using TOC measurements. However, using the geometric mean of the measured TOC values from 0h until 72h only approximately 11 % evaporated from the test solutions. Therefore, the EC50 values were not corrected.

Test condition: A static test was performed.
Inoculum density: approx. 10,000 cells/ml
Determination of cell density after 0, 24, 48, 72, and 96 h (fluorescence at 685 nm).
Duration of test: 96h
Test temperature: 21 - 25 °C
Test vessel: Erlenmeyer flask (nominal volume : 250 ml)
Test volume: 100 ml
Initial pH: approx. 8.0
Illumination: artificial light, permanent
Illumination intensity: 120 µE/m2s

Nominal test concentrations: 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 mg/l.

Reliability: (1) valid without restriction
Test method comparable to guideline study, in accordance with accepted national standards

Flag: Critical study for SIDS endpoint

26-JAN-2004 (39) (40) (36)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: activated sludge, domestic
Exposure period: 1 hour(s)

Unit: mg/l **Analytical monitoring:** no
EC50: > 1000
EC20 : ca. 35

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year: 1993
GLP: yes

Remark: The EC20 in the activated sludge respiration inhibition test is below 100 mg/l. Depending on local conditions and existing concentrations, disturbances in the biodegradation process of activated sludge are possible.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
25-MAY-2003 (41)

Type: aquatic
Species: activated sludge, industrial
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC20 : > 1000

Method: Directive 88/302/EEC, C.11
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: No inhibition of respiration up to 1000 mg/l could be observed.
No dysfunction of adapted biological WWTPs are expected when operated properly.
Test condition: Inoculum: 1 g/l dry mass (activated sludge of BASF WWTP)
Test temperature: 20 °C
Reliability: (2) valid with restrictions
Test method comparable to guideline study, in accordance with accepted national standards
01-JUL-2003 (42)

Type: aquatic
Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 106
EC20 : = 72.1
EC80 : = 211

Method: other: Bioluminescence Assay
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Results after 15 min were as follows:
EC20 = 84 mg/l
EC50 = 148 mg/l
EC80 = 271 mg/l.

Reliability: (4) not assignable
Manufacturer/producer data without proof, only short communication, neither raw data nor report available

01-JUL-2003 (43)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 17 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 84
EC50: = 178
EC90 : = 237

Method: other: Pseudomonas Cell-Multiplication Inhibition Assay according to DIN 38412, part 8 (Draft; Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Bakterien)
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test condition: Test strain: Ps. putida DSM 50026
Test temperature: 20 °C
Pre-culture: 7 h;
Test volume: 10 ml.

Nominal test concentrations: 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 with 4 parallel incubations per concentration.
Reliability: (1) valid without restriction
Test method comparable to guideline study, in accordance with accepted national standards
Flag: Critical study for SIDS endpoint

01-JUL-2003 (44)

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 17 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 124
EC50: = 212
EC90 : = 296

Method: other: Pseudomonas Cell-Multiplication Inhibition Assay, DIN 38412, Part 8 (Draft), Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Bakterien
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test condition: Test strain: Ps. putida DSM 50026
Test temperature: 20 °C; pre-culture: 7 h;
test volume: 10 ml.

Nominal test concentrations:
9.01, 18.03, 39.06, 78.12, 156.25, 312.5, 625, 1250, 2500, 5000, 10000 with 4 parallel incubations per concentration.
Reliability: (1) valid without restriction
Test method comparable to guideline study, in accordance with accepted national standards

25-MAY-2003 (45)

Type: aquatic
Species: Tetrahymena pyriformis (Protozoa)
Exposure period: 40 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 68.1

Method: other: 40-h impairment growth assay (TETRATOX)
Year: 1997
GLP: no
Test substance: no data

Method: In this 40-h impairment growth assay the population density is quantitated spectrometrically with absorbence at 540 nM. Test conditions allowed for 8 - 9 cell cycles in controls. The 50 % growth inhibitory concentration (IGC50) can be determined and corresponds to an EC50.
Reliability: (2) valid with restrictions
well documented publication which meets basic scientific principles
Flag: Critical study for SIDS endpoint
09-JUL-2003 (46)

Type: other: antimicrobial activity
Species: other fungi: Penicillium chrysogenum
Exposure period: 120 hour(s)
Unit: mg/l **Analytical monitoring:** no
MIC or EC100 : = 50

Method: other: antimicrobial activity testing
GLP: no

Method: The n-hexane extract of dried T. balsamita flowers was separated, the active fractions were combined and divided into distillate and these distillates subsequently were evaluated for antimicrobial activity.

The antimicrobial activity of the identified components from the distillate on diverse bacteria and fungi was regarded. The Minimum Inhibitory Concentration (MIC) was calculated. As highest test concentration 800 µg/mL was chosen.
Reliability: (3) invalid
method not validated
01-JUL-2003 (47)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 60
Vehicle: CMC
Doses: 316, 464, 562, 681, 825, 1000 mg/kg bw
Value: = 690 mg/kg bw

Method: other: BASF method
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Comparable to former OECD 401
Result: MORTALITY
Deaths occurred within 1 hr at 1000 mg/kg bw and 681 mg/kg bw or within 1 d after doses of 562, 681, 825 mg/kg bw.
Mortalities were as follows:
0/10 animals at 316 or 464 mg/kg bw;
2/10 animals at 562 mg/kg bw; 3/10 animals at 681 mg/kg bw;
9/10 animals at 825 mg/kg bw;
10/10 animals at 1000 mg/kg bw.
Calculated LD50 value (95% confidence intervals) for male and female rat was 690 (629-758) mg/kg bw (probit analysis according to Finney).

CLINICAL SIGNS
Poor condition, salivation, apathy, dyspnoea was seen in all animals at 316 mg/kg bw and above. Labored breathing, unsteady gait, tremors was seen from 464 mg/kg bw onwards. Unkempt fur, reddening of the skin, lacrimation were seen at 562 mg/kg bw and above; spasms were noted at 1000 mg/kg bw.

NECROPSY FINDINGS
Acute heart dilatation on both organ sides, peripheral lobular pattern in liver, glandular stomach reddened in animals that died. In survivors forestomach wall was thickened; forestomach clotted with liver, spleen and peritoneum.
Test condition: TEST ORGANISMS
Dose groups contained 5 male and 5 female rats. Mean group body weights ranged between 190-260 g in males and 160-190 g in females.

ADMINISTRATION
Test substance was given by gavage, dissolved in 0.5% CMC in a constant volume of 10 ml/kg bw.
EXAMINATIONS
Animals were inspected daily for signs of pharmacologic or toxicologic effects during a 14 d observation period. Body weight was measured before dosing, and at days 2-4, 7 and 14

thereafter. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.
CALCULATION METHOD OF LD50
Probit analysis according to Finney

Test substance: Substance no. 78/452, purity ca. 98%

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

Flag: Critical study for SIDS endpoint

14-APR-2003 (48)

Type: LD50
Species: rat
Strain: other: Gassner
Sex: male/female
No. of Animals: 120
Vehicle: CMC
Doses: 0.4, 0.8, 1.0 1.25, 3.2 and 6.4 ml/kg bw (ca. 352, 704, 880, 1100, 2816 and 5632 mg/kg bw)
Value: 810 mg/kg bw

Method: other: BASF method
Year: 1971
GLP: no
Test substance: other TS

Result: MORTALITY
0.4 ml/kg bw: no deaths
0.8 ml/kg bw: 0/10 males and 4/10 females died within 24 hrs
1.0 ml/kg bw: 6/10 males and 7/10 females died within 24 hrs
1.25 ml/kg bw: 8/10 males and 10/10 females died within 24 hrs
3.2 ml/kg bw: 10/10 males and 10/10 females died within 60 min
6.4 ml/kg bw: 10/10 males and 10/10 females died within 60 min

CLINICAL SIGNS
Convulsions, dyspnoea, atonia, abdominal position.

NECROPSY
Hyperemia in liver, watery stomach contents, atonic intestines. Necropsy was performed by a pathologist.

LD50 after 7 days was 920 µl/kg bw (calculated according to Litchfield and Wilcoxon), corresponding to 810 mg/kg bw.

Test condition: TEST ORGANISM

10 male and 10 female per dose; no vehicle controls.
Average weight at beginning of the test:
140 - 260 g (females), 170 - 280 g (males),
ADMINISTRATION
Gavage application in water containing CMC supplemented with 2-3 drops of Cremophor (as solubilizer). The doses above were administered at concentrations of 4, 8, 10, and 30% (v/v) aqueous emulsions, respectively.
EXAMINATION
Observation period 7 days. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.

Test substance: Substance no. XXI/56. 80% 3-methyl-2-butenal, 20%

3-methylbut-3-en-1-ol
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
14-APR-2003 (49)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Value: 700 mg/kg bw

Method: other
Year: 1979
GLP: no
Test substance: other TS

Test substance: 3-methyl-2-butenal, purity not mentioned
Reliability: (4) not assignable
Secondary literature
14-APR-2003 (50)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 100
Doses: 0.97, 3.15, 3.59, 3.88, 6.08 mg/l (970, 3150, 3590, 3880, 6080 mg/m3)
Exposure time: 4 hour(s)
Value: = 3.7 mg/l

Method: other
Year: 1979
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Method: Similar to OECD 403
Result: MORTALITY
LC50-values were calculated from the data below as follows: 3.5, 4.0, and 3.7 mg/l (3500, 4000 and 3700 mg/m3) for male, female, and combined male and females rats, respectively.

Dose	Dead animals/out of total
0.97 mg/l	0/20
3.15 mg/l	0/20
3.59 mg/l	9/20
3.88 mg/l	13/20
6.08 mg/l	20/20

CLINICAL SIGNS
Animals from the 2 lowest dose groups recovered within 5 days from signs which included lacrimation and nasal discharges, closed lids, labored breathing, unkempt fur. Signs were more pronounced in animals receiving higher doses and included dyspnoea, unsteady gait, stagger, apathia,

tremors, opacity of cornea. Deaths occurred within 5 days after exposure. Survivors were not free of symptoms when sacrificed at the end of the 14 day observation period. Mean group body weights were reduced in a dose-dependent manner in both sexes 7 days after dosing. 14 days after dosing body weights of female survivors were not different from controls. The same applied for the males except for those receiving 3.88 mg/l. Mean body weight of this group was reduced by approx. 11% when compared with control animals.

NECROPSY FINDINGS
Acute heart dilatation in atrei and acute congestion. Several victims showed peripheral lobular pattern in liver and edematous lungs. No changes were seen in survivors.

Test substance: 3-methyl-2-butenal; substance no. 78/452, ca. 98% purity

Conclusion: Combined male and female LC50 was 3.7 mg/l (3700 mg/m3). LC50 was comparable in males and females (LC50 males 3.5 mg/l, LC50 females 4.0 mg/l).
Due to irritancy vapors affected skin, respiratory tract and cornea of the test animals. Symptoms persisted until the end of the 14 day observation period.

Test condition: TEST ORGANISMS
10 Sprague Dawley rats per sex and dose, of weight range 185 +/- 15 g, were used. No control animals were included.
ADMINISTRATION
Animals were placed in a 200 l whole body exposure chamber. Animals were exposed for 4 hrs. The dynamic inhalation atmospheres generation involved constant delivery of the test substance to an evaporator (78°C) situated in an air stream.
EXAMINATIONS
During the exposure samples of chamber air were taken for measurement of the test substance. Animals were observed during exposure and during a 14 day observation period for signs of pharmacologic or toxicologic effects. Body weight was measured before exposure, and at 7 and 14 days thereafter. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.

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

Flag: Critical study for SIDS endpoint

15-APR-2003 (51)

Type: other: Inhalation Risk Test (IRT)
Species: rat
Strain: Sprague-Dawley
Sex: female
No. of Animals: 24
Doses: saturated atmosphere (28-44 mg/l; 28000-44000 mg/m3)
Exposure time: 1 hour(s)

Method: other
Year: 1971
GLP: no
Test substance: other TS

Method: Inhalation Risk Test according to Smyth, H.F. et al., Am. Ind. Hyg. Ass. J., 23, 95-107 (1962)
Remark: No calculation of LC50.

Result: MORTALITIES
After 10 min exposure at 27.78 mg/l: no death (0/12 animals) was noted within the 7 d observation period.
30 min exposure at 43.8 mg/l: 6/6 animals died. 2/6 during the exposure; 2/6 after exposure; 2/6 one or two days after exposure.
60 min exposure time at 35.1 mg/l: 6/6 animals died during the exposure period.

CLINICAL SIGNS
10 min exposure at 27.78 mg/l: Heavy attempts to escape, eye irritations, dyspnoea. Noisy breathing resolved overnight.
30 min exposure at 43.8 mg/l: Heavy attempts to escape, eye irritations, dyspnoea, labored breathing. Necrosis of corneae, nostrils, extremities.
60 min exposure at 35.1 mg/l: Heavy attempts to escape, eye irritations, dyspnoea, labored breathing. Dark brown eyes.

NECROPSY
No abnormal findings except 2 cases of pneumonia of the middle lobule in short term exposed animals.

Test substance: Substance no. XXI/56. 80% 3-methyl-2-butenal, 20% 3-methylbut-3-en-1-ol

Test condition: TEST ORGANISMS
24 female rats.
ADMINISTRATION

6 animals each were exposed to an atmosphere saturated with the test substance at 20° C. Atmospheres were created by blowing air (200 l/h) through a layer (5 cm) of the test substance.
Nominal substance concentrations were calculated to be 27.78 mg/l (10 min.), 43.8 mg/l (30 min.) and 35.1 mg/l (60 min.) by using the weight loss of test substance and the amount of air used during exposure.
EXAMINATIONS
Observation period was 7 days. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.

Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions

14-APR-2003 (49)

Type: other: Inhalation Risk Test (IRT)
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 24
Doses: enriched atmosphere (21-24 mg/l; 21000-24000 mg/m3)
Exposure time: 1 hour(s)

Method: other
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Inhalation Risk Test according to Smyth, H.F. et al., Am. Ind. Hyg. Ass. J., 23, 95-107 (1962)
Result: MORTALITIES

After 10 min exposure at 24.32 mg/l: no death (0/12 animals) was noted within the 7 d observation period.
30 min exposure at 22.10 mg/l: 1/6 animals died.
60 min exposure time at 21.2 mg/l: 6/6 animals died.

No LC50 was calculated.

CLINICAL SIGNS
Heavy attempts to escape, eye irritations, eyelid closure, salivation, lacrimation, dyspnoea. Stagger, unsteady gait, spasms, lateral posture.

NECROPSY
Distended lungs in animals which died. Survivors without findings.

Test substance: 3-methy-2-butenal. Substance no. 78/452, purity ca. 98%.

Test condition: TEST ORGANISMS
24 rats.
ADMINISTRATION
6 animals each were exposed to an atmosphere saturated with the test substance at 20° C. Atmospheres were created by blowing air (200 l/h) through a layer (5 cm) of the test substance.
Nominal substance concentrations were calculated to be 24.32 mg/l (10 min.), 22.10 mg/l (30 min.) and 21.2 mg/l (60 min.) by using the weight loss of test substance and the amount of air used during exposure.
EXAMINATIONS
Observation period was 7 days. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.

Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions

Flag: Critical study for SIDS endpoint

14-APR-2003 (48)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 62
Vehicle: other: none
Doses: 1200, 1600, 2000, 2500, 3200, 4000 mg/kg bw
Value: 3400 mg/kg bw

Method: other: BASF method
Year: 1978
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Comparable to OECD 402
Result: MORTALITY
Deaths occurred within 7 days after dosing, predominantly during the first two days. LD50 of 3400 mg/kg bw was calculated from the following data (probit analysis according to Finney):

1200 m/kg bw:
0/5 males died after 14 days, 1/5 female died after 24 hrs
1600 mg/kg bw:
0/5 males died after 14 days, 3/5 females died after 24 hrs, 1 additional female after 7 days
2000 mg/kg bw:
3/6 males died after 24 hrs, 1 additional male after 7 days
0/6 females died 14 days
2500 mg/kg bw:
1/5 males died after 24 hrs, 1 additional male after 7 days, 1/5 females died after 7 days
3200 mg/kg bw:
3/5 males died after 24 hrs, 1 additional male after 48 hrs, 2/5 females died after 24 hrs, 1 additional female after 14 days
4000 mg/kg bw:
3/5 males died after 7 days, 1/5 females died after 7 days

CLINICAL SIGNS
Poor condition, apathy, dyspnoea, stagger, unsteady gait, abdominal position, paresis was seen in dosed animals.
Local skin effects after 24 hrs comprised substance-induced staining and intense swelling, and necrosis after 48 hrs.

NECROPSY FINDINGS
Acute heart dilatation on both sides of the organ and acute heart congestion. Peripheral lobular pattern in liver in some victims. No changes noted in survivors.

Test condition: TEST ORGANISMS
Generally 5 rats per sex and dose were used; 6 animals per sex at 2000 mg/kg bw. Mean body weight was 218 g (males) and 186 g (females).
ADMINISTRATION
Undiluted test substance was applied to dorsal skin. Size of area was approx. 42 cm2. Observation period 14 days.
EXAMINATIONS
Animals were observed daily during a 14 day observation period for signs of pharmacologic or toxicologic effects. Body weight was measured before exposure. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.
LD50 was calculated using probit-analysis according to Finney - lognormal distribution.

Test substance: 3-methyl-2-butenal; substance no. 78/452. Purity ca. 98%

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

Flag: Critical study for SIDS endpoint

15-APR-2003 (48)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain: NMRI
Sex: male/female
No. of Animals: 20
Vehicle: CMC
Doses: 200, 700 mg/kg bw
Route of admin.: i.p.
Value: >= 200 - 700 mg/kg bw

Method: other: BASF-method
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Intraperitoneal injection of test substance at 2 dose levels.
Result: MORTALITY
0/10 animals died at 200 mg/kg bw; 10/10 animals died at 700 mg/kg bw within 1 day after dosing.

CLINICAL SIGNS
Poor condition, apathy, dyspnoea in all dosed animals. Stagger, unsteady gait, unkempt fur was seen low dose animals. Spasms and abnormal position in high dose animals.

NECROPSY FINDINGS
No abdominal test substance precipitation or clotting in victims or survivors noted.
Test condition: TEST ORGANISMS
5 mice per sex and dose. Mean body weight 25 g in males, 22 g in females.
ADMINISTRATION
Test substance was given by i.p. injection, emulsified in 0.5% CMC, in a constant volume of 10 ml/kg bw.
EXAMINATIONS
Animals were inspected daily for signs of pharmacologic or toxicologic effects during a 14 day observation period. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.
Test substance: 3-methyl-2-butenal; substance no. 78/452. Purity ca. 98%
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
14-APR-2003 (48)

Type: LD50
Species: mouse
Strain: other: Kisslegg mice
Sex: male/female
No. of Animals: 60
Vehicle: CMC
Doses: 250, 320, 400, 500, 640, 800 µl/kg bw (ca. 220, 282, 352, 440, 563, 704 mg/kg bw)
Route of admin.: i.p.
Value: ca. 378 mg/kg bw

Method: other
Year: 1971
GLP: no
Test substance: other TS

Result: MORTALITY
Deaths occurred within 14 days after dosing, predominantly during the first two days. LD50 of 430 µl/kg (corresponding to 378 mg/kg) was estimated from the following data (calculation method not mentioned in the report):

Dose (µl/kg)	Deaths/out of number of animals
250	1/10

320 1/10
400 4/10
500 7/10
640 9/10
800 10/10

CLINICAL SIGNS
Symptoms observed immediately after high dose injections (400-800 µl/kg) included enhanced and spastic breathing, lateral position with spasms, salivation. Atony was seen after 2 hrs which persisted for several days. Animals were free of symptoms on days 8 through 13.

NECROPSY FINDINGS
Abdominal adhesions, intestines in some animals atonic.

Test condition: TEST ORGANISMS
5 mice per sex and dose were used.
ADMINISTRATION
Test substance was given i.p. as an 8% emulsion in CMC.
EXAMINATIONS
Animals were observed daily during a 14 day observation period for signs of pharmacologic or toxicologic effects. At the end of the observation period survivors were sacrificed and necropsied as were animals that died.

Test substance: Substance no. XXI/56. 80% 3-methyl-2-butenal, 20% 3-methylbut-3-en-1-ol

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

14-APR-2003 (49)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.p.
Value: 700 mg/kg bw

Method: other
Year: 1979
GLP: no
Test substance: other TS

Test substance: 3-methyl-2-butenal, purity not mentioned
Reliability: (4) not assignable
Secondary literature

11-APR-2003 (50)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 4 hour(s)

No. of Animals: 6
Result: corrosive

Method: other: BASF-method
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: MORTALITY
None

EFFECTS TO SKIN
3 minutes exposure period:
Immediately after the 3 min exposure period no edema was seen whereas erythema could not be assessed due to substance-induced staining of the skin. During the examinations at 1, 2 and 8 days after the treatment very slight erythema, but no edema were seen.

60 minutes exposure period:
Immediately after the 60 min exposure period very distinct edema were seen in both animals which persisted at 48 hrs. After 8 days slight edema were noted.

Immediately after the 60 min exposure period erythema could not be assessed due to substance-induced staining of the skin in one animal, but very distinct erythema and soft necrosis was noted in the other. Very distinct erythema and necrosis was seen in both animals at 1, 2, and 8 days after the treatment. With time the necrotic skin hardened and became leather-like. Necrosis was confirmed during the pathological examination of the skin.

240 minutes exposure period:
After the 240 min exposure period distinct to very distinct edema were seen in both animals which persisted at 48 hours. After 8 days slight edema were noted.
Immediately after the 240 min exposure period distinct erythema were noted which persisted and aggravated until day 8 after treatment. Necrosis was seen 24 hours after treatment
and persisted until the end of the observation period on day 8. The necrotic skin became leather-like with time. Necrosis was confirmed during the pathological examination of the skin.

Test condition: ADMINISTRATION
Undiluted test substance was applied to the skin of 2 animals each for 3, 60, or 240 minutes.
EXAMINATIONS
Examination of the skin at the site of application at the end of the exposure and 24, 48, 192 hours after treatment. Pathologic examination of the skin after sacrifice of the test animals.

Test substance: 3-methyl-2-butenal; substance no. 78/452. Purity ca. 98%

Conclusion: Edema, erythema and necrosis of the skin were seen as early as 1 h after treatment and persisted until the end of the observation period on day 8.

Based on this finding no more eye irritation test was performed.

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
Flag: Critical study for SIDS endpoint
14-APR-2003 (48)

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 20 hour(s)
No. of Animals: 2
Result: corrosive

Method: other: BASF-method
Year: 1971
GLP: no
Test substance: other TS

Result: MORTALITY
None

EFFECTS TO SKIN
Exposure of the ear and dorsal skin caused erythema, edema and necrosis which persisted at the end of the observation period, 8 days after treatment. Severity correlated with the length of the exposure period as outlined in the table (cf. attached document). Necrosis was seen after 20 hrs exposure period.
Test condition: TEST ORGANISMS
Female White Vienna rabbits, body weight ca. 3-3.5 kg.
ADMINISTRATION
Undiluted test substance was applied under occlusive conditions to the dorsal skin and to the ear. Exposure time was 1, 5 and 15 min (dorsal skin) and 20 hrs (dorsal skin and ear).
EXAMINATIONS
Examinations of the skin at 24 hrs and 8 days after application.
Test substance: Substance no. XXI/56. 80% 3-methyl-2-butenal, 20% 3-methylbut-3-en-1-ol
Attached doc.: Skin irritation.doc
Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions
14-APR-2003 (49)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .05 ml
Comment: not rinsed
No. of Animals: 2
Result: highly irritating

Method: other: BASF-method
Year: 1971
GLP: no
Test substance: other TS

Remark: Deficiencies compared with guideline are acceptable since

	the potential of severe eye lesions is also expected from the corrosive effects of the TS on skin.
Result:	Finding after 1 hr Slight erythema, very distinct edema, distinct corneal opacity was seen. Findings after 24 hrs Erythema, edema, opacity persisted. Hemorrhages. Findings after 8 days Distinct erythema, slight edema, persistent distinct opacity. Ingrown blood vessels, staphyloma.
Test condition:	TEST ORGANISMS Two White Vienna rabbits, one male and female each, mean body weight 2.66 kg. ADMINISTRATION Instillation of 50 µl (44 mg) of undiluted test substance into the rabbit eye. EXAMINATIONS Examination after 1 and 24 hrs, and after 8 days.
Test substance:	Substance no. XXI/56. 80% 3-methyl-2-butenal, 20% 3-methylbut-3-en-1-ol
Conclusion:	Severe eye irritation, staphyloma. Persistent corneal opacity. Risk of persistent severe eye lesions upon contact to the eyes.
Reliability:	(2) valid with restrictions Comparable to guideline study with acceptable restrictions
Flag:	Critical study for SIDS endpoint

14-APR-2003 (49)

5.3 Sensitization

Type:	Guinea pig maximization test	
Species:	guinea pig	
Concentration 1st:	Induction	.2 % active substance intracutaneous
2nd:	Induction	10 % active substance occlusive epicutaneous
3rd:	Challenge	5 % active substance occlusive epicutaneous
No. of Animals:	20	
Vehicle:	water	
Result:	sensitizing	
Classification:	sensitizing	

Method:	Directive 84/449/EEC, B.6 "Acute toxicity (skin sensitization)"
Year:	1991
GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4
Result:	Mortalities: One animal of the control group was found dead on day 8 after application. Skin effects: (1) Well defined erythema and slight edema were seen at the injection sites of Freund's complete adjuvans (FCA)/saline (1:1) in both test and control animals. Well defined erythema and slight edema were also seen at the injection sites of the test substance in FCA/saline in test animals. (2) Well defined erythema, slight edema and incrustation (partially open from interadermal injections) were noted after percutaneous induction in the test animals.

(3) Positive skin reactions, i.e. erythema, were observed in 3/9 test animals after the challenge procedure at both readings. No reaction (0/5 animals) was seen in the control group. The positive control substance led to positive skin reactions (erythema) in 10/10 animals at both readings after the challenge.

Test condition: TEST ANIMALS
20 female Albino Dunkin Hartley Guinea pigs were used (10 test animals, 2 control groups 5 animals each), body weight range 300-350 g.

ADMINISTRATION/EXPOSURE
1. Pretests: range finding
After two 24-hr percutaneous occlusive application within 96 hrs the minimum irritant concentration was found to be 10% and the maximum non-irritant concentration 5% in aqua bidest. Based on these results test substance was used during percutaneous induction as a 10% solution, and in 5% concentration during percutaneous challenge.

2. Main study
Induction:
Injections: The test animals received three pairs of intradermal injections (0.1 ml each; FCA/saline, 0.2% test substance in saline, 0.2% test substance in FCA/saline 1:1). 5 control animals were similarly treated receiving vehicle only without test substance. Epidermal application: One week later patches of filter paper saturated with 0.3 g of the test substance formulation in aqua bidest were placed over the injection sites and fixed. The occlusive dressings were left in place for 48 hrs. The control group animals remained untreated. Reaction sites were assessed 48 hrs after the beginning of application.
Challenge:
Three wks after the epidermal induction test and control animals were subjected to the challenge procedure. Hair was clipped and shaved, and a filter paper patch saturated with the test substance formulation was applied to the flank using the same method as for the epidermal application. The animals were exposed to about 0.15 g of the substance. The dressings were removed after 24 hrs, and the treated sites were assessed for erythema and oedema. Readings were 24 and 48 hrs after the removal of the patch.
A positive control test was performed with 1-chloro-2,4-dinitrobenzene using another 20 animals.

EXAMINATIONS
Erythema and oedema reactions were assessed at 24 and 48 hrs after bandage removal of the epidermal application and the challenge procedure using the 0-4 numerical scoring system according to Draize. Body weights of the main test animals was determined during acclimatization and on days 1 and 28 of the test period.
Stability of the test substance in vehicle was examined.

EVALUATION
According to the evaluation criteria of the Directive 83/467 EEC the evaluation "sensitizing" results if at least 30% of the test animals show skin reactions.

Test substance: 3-methyl-2-butenal; substance no. 90/734, purity 96.3%

Conclusion: 3-methyl-2-butenal had a slight, borderline sensitizing effect in the guinea pig maximation test.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
14-APR-2003 (52)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: inhalation
Exposure period: 28 d/ 20 exposures
Frequency of treatment: 5 d/wk
Post exposure period: none
Doses: 0, 30, 100, 300 ppm (vapor); corresponds to 0, 0.10, 0.34, and 1.02 mg/l (0, 100, 340, 1020 mg/m3)
Control Group: yes, concurrent vehicle
NOAEL: = 30 ppm
NOAEC for systemic toxicity :
= 300 ppm

Method: OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Substance concentrations were 0, 30.3, 100.6 and 298.9 ppm corresponding to 0, 0.10, 0.34, and 1.02 mg 3-methyl-2-butenal/l (0, 100, 340, 1020 mg/m3).

(1) Findings in the low dose 30 ppm test group (0.10 mg/l)
Clinical signs were snout wiping, restlessness, eyelid closure at the beginning of the exposure indicating slight, transient sensory irritation. No influence on body weight was noted. No effects on hematological and clinicochemical parameters or urinalysis were noted. No pathological changes noted.

(2) Findings in the intermediate dose 100 ppm test group (0.34 mg/l)
At the beginning of the exposure snout wiping, restlessness, eyelid closure, salivation, ruffled fur, apathy were seen. Animals accustomed to the exposure during the first week and only restlessness and ruffled fur persisted further on. No influence on body weight development was noted. No effects on hematological and clinicochemical parameters or urinalysis were noted.
The absolute testes weights were significantly decreased in males (-7.5%). No histopathological changes were observed in the testes.
In the respiratory tract, histopathology revealed focal metaplasia of respiratory epithelium into squamous epithelium in the nasal cavity of all males and 3 females, and in the cartilage of the larynx in two males and one female.
No other pathological or microscopical changes were noted.

(3) Findings in the high dose 300 ppm test group (1.02 mg/l)
Clear signs of irritation over the entire exposure period such as eyelid closure, salivation, ruffled fur, apathy. The symptoms disappeared rapidly after each exposure. Body weight development was significantly retarded in males (-59.8% compared to controls, days 0 - 28) and slightly impaired in females (-39.7% compared to controls, days 0 - 28). No ophthalmologic changes were detected.
Increased number of polymorphonuclear neutrophils accompanied by a decrease in leucocytes in females was interpreted as a sign of inflammation in the respiratory tract.
No effects on urinalysis were noted.
Gross pathology revealed a significantly decreased terminal body weight in males (-14.1%). The absolute testes weights were significantly decreased (-6.1%) whereas the relative testes weights were significantly increased (+12.1%). No treatment related histopathological changes were observed in this organ.
Histopathology revealed purulent inflammation in the nasal cavity of all males and some females. Focal metaplasia of respiratory epithelium into squamous epithelium in the nasal cavity and in larynx of all males and site specifically in all or some females. In one male and one female atrophy of the olfactory epithelium (level II) was noted.

Test condition: TEST ORGANISMS
SPF-Wistar-rats/chbb:THOM, supplied by Dr. K. Thomae GmbH, Biberach (FRG). The age on delivery was about 7 weeks.
40 animals were used in the main test, i.e. 5 animals per dose and sex. Mean group body weights ranged from 203-216 g in males and from 165-169 g in females.

ADMINISTRATION/EXPOSURE
Animals were subjected to a total of 20 exposures (5 per week), 6 hrs each at nominal concentrations of 0, 30, 100 and 300 ppm (= 0, 0.10, 0.34, and 1.02 mg/l or 0, 100, 340, and 1020 mg/m3). Exposure took place in a whole body inhalation chamber, volume 1100 l, to which the animals had been accustomed during a 5-d preflow period. Animals were kept singly in wire cages. Dynamic atmosphere generation involved the use of a heated evaporator kept at 40°C. Supply air flow was ca. 22 m^3/h.
EXAMINATIONS
Analytical
Air flow, temperature, and pressure conditions were continuously recorded and regulated by an automated measuring system. Nominal test substance concentrations were calculated. Mean daily actual concentrations were calculated from several daily measurements of exposure concentrations.
Animals
Body weights were determined at -5 and -1 day before beginning of the exposure and weekly thereafter. Behavior and clinical signs were checked thrice at exposure days and once on workdays during the preflow period and the post-exposure observation day. Ophthalmoscopical examination of all animals was performed before the preflow period; at the end of the study all control and high dose animals were examined.
Clinical chemistry, hematology and urinalysis were carried out in a randomized sequence. Urine was collected overnight

in single animal metabolism cages. Parameters included:
(1) Hematology: leukocytes, erythrocytes, hemoglobin, hematokrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular mean hemoglobin concentration, platelets, differential blood count, reticulocytes, clotting analysis.
(2) Clinical chemistry: transaminases (ALT, ASP), alkaline phosphatase, serum g-GT, inorganics (Na, K, Ca, Mg, Cl, PO4), urea, creatinine, glucose, bilirubin, total protein, albumin, globulins, triglycerides and cholesterol.
(3) Urinalysis: nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood; specific gravity, sediment, color, turbidity, volume.
Necropsy included weighing of selected organs (liver, kidneys, adrenal glands, lungs, testes) and gross pathology in all animals. Histopathology of nasal cavity, pharynx/larynx, trachea, lungs, mediastinal lymph nodes, adrenal glands and testes was performed in all exposure levels, liver, spleen, kidney and heart were examined in all control and high dose animals.

Non-parametric one-way analysis using KRUSKALL-WALLIS test was used to evaluate body weight and clinical data. pathology data. If p-values were equal or less than 0.05, the MANN-WHITNEY U-test test was also used for pairwise comparison of each dose group with the control group. FISHER's exact test was used for pairwise comparison of urinalysis of each dose group with the control group.

Test substance: 3-methyl-2-butenal; substance no. 90/734-1; purity 98.29%

Conclusion: The clinical findings at low concentrations during the first week of exposure were not interpreted as toxic effects but were attributed to the marked odor of the test substance.

Irritation of the upper respiratory tract was seen after repeated exposure (4 weeks, 5d/wk, 6 hrs/d) at or above 100 ppm 3-methyl-2-butenal (=0.34 mg/l) as evidenced by the concentration dependend focal metaplasia of respiratory epithelium into squamous epithelium. Additionally, atrophy of the olfactory epithelium was noted in 2/10 animals at 300 ppm.

No ophthalmologic changes were seen up to and including 300 ppm.

No systemic toxicity was seen at concentrations up to 300 ppm (= 1.02 mg/l). The observed decreases in body weights were assigned to the discomfort of the purulent rhinitis found in all high dose male and some female animals.

A substance related effect for the significantly decreased absolute testes weights at 100 ppm and 300 ppm and the significantly increased relative testes weights at 300 ppm was regarded as unlikely as there were no histopathological correlates in this organ. At least for dose group 3 (300 ppm), the weight changes seemed to be a consequence of the reduction of the terminal body weight.

Therefore, the NOAEC for local irritation was 30 ppm, the LOAEC for local irritation of the upper respiratory tract was judged to be 100 ppm. For systemic toxicity the NOAEC

was 300 ppm.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
04-MAR-2004 (53)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: drinking water
Exposure period: 18 wk
Frequency of treatment: 7 days per week
Post exposure period: none
Doses: 0, 50, 200, 800 ppm (ca. 0, 6, 21, 77 mg/kg bw/d)
Control Group: yes, concurrent no treatment
NOAEL: ca. 77 mg/kg bw
NOEL (water and food intake) :
ca. 21 mg/kg bw

Method: other: OECD 415
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Study was conducted in accordance with OECD 415
Remark: This summary is limited to parental repeated dose toxicity. The full study summary includes fetal data and is contained in chapter 5.8.1.
Result: TS in drinking water
Stability and homogeneity was demonstrated.

TS intake: Calculated intake at dose levels 50, 200, and 800 ppm in drinking water was

F0 males: 4.9, 19.0, 72.0 mg/kg bw/d (premating)
F0 females: 6.4, 23.5, 81.8 mg/kg bw/d (premating)
F0 females: 6.2, 23.2, 82.0 mg/kg bw/d (gestation)
F0 females: 8.3, 31.9, 123.5 mg/kg bw/d (lactation)

The mean dose administered for both sexes during premating was ca. 5.7 mg/kg bw/d, ca. 21.3 mg/kg bw/d, and ca. 76.9 mg/kg bw/d in the low, mid and high dose groups, respectively.

Mortality
One high dose female was found dead during premating wk 10. Erosions/ulceration of glandular stomach mucosa was found at necropsy, but this was assessed as being not causative for death. No other mortalities occurred.

CLINICAL OBSERVATIONS
Clinical findings: No substance-related signs or any disturbance of behavior noted in all male and female F0 animals during the entire administration period.
Water consumption: Unchanged at the low and intermediate dose level, significantly reduced during premating (-20% males, -24% females) and in females during gestation (-17%) and lactation (-10%). This was regarded as a substance-related effect, probably due to bad taste and/or smell of TS solutions.

Food consumption: Significantly reduced in high dose males during the first weeks of treatment (wk 0-4), and similar to control animals thereafter. No other relevant changes noted.

Body weight, body weight gain: Not influenced by TS in any group at any time interval.
Pathology:
Gross lesions were noted in glandular stomach (erosion/ulcer), cecum, liver, testes (size reduced) epididymides (size reduced) oviducts (cyst), uterus (cyst), eyes and skin. However, these were single observations or distributed over control and treated groups without evidence of relationship to treatment. The same applied to histopathology.
Overall, no substance-related adverse effects were noted in F0 parental animals in groups at 50 and 200 ppm.

Changes noted in F0 animals at 800 ppm were a decreased water intake in both sexes and a transient decrease of food intake of males during the first 4 weeks of treatment. There were no other differences in the high dose group F0 animals compared to controls.

Test condition: TEST ANIMALS
25 male and female Wistar rats (CrlGlxBrlHan:Wi) were used per dose level. At the beginning of treatment, animals were 34+/- 1 days old. Mean body weights were 107 (95-117)g for male and 96 (86-105)g for female animals. During the study period the animals were housed singly.
OVERALL EXPERIMENTAL DESIGN
After acclimatization F0 animals were treated until day 21 after parturition of their progeny. Animals were mated 76 days after beginning of treatment and allowed to rear their F1 pups until day 21 after parturition. Then all F0 and F1 animals were sacrificed and examined.

EXPOSURE/ADMINISTRATION
Animals received TS in the drinking water at concentrations of 0, 50, 200, and 800 ppm.
EXAMINATIONS
TS stability for 4 days was examined. Homogeneity was given by complete water solubility. Concentration control analyses were performed at start, middle and end of the treatment period.
Parental animals were observed daily during the study. Observations and examinations included: Daily check for signs of toxicity and mortality. Water consumption was determined once a week during premating for 3-d periods, and for females on days 0-1, 6-7, 13-14, and 19-20 post coitum during gestation, and on days 1-2, 4-5, 7-8, and 14-15 after parturition. Food consumption was determined weekly. After 10th week in pregnant females weekly during gestation, days 1-4, 4-7, and 7-14 during lactation.
Body weight data of F0 animals: once a week at the same time of the day

Pathology:
Necropsy:
Animals were necropsied and assessed by gross pathology. No weight parameters were determined.
Histopathology:

Of the F0 generation parental animals, the following organs or tissues were fixed in 4% formaldehyde solution or in BOUIN's solution. After sufficient fixation, the organs fixed in BOUIN's solution were embedded in paraplast: vagina, cervix uteri, uterus, ovaries, testes, epididymides, seminal vesicles, coagulating glands, prostate gland, pituitary gland, all gross lesions.
After the organs were fixed, histotechnical processing, examination by light microscopy and assessment of findings was performed.

STATISTICAL DATA EVALUATIONS
DUNNETT's test was used to compare treated and control groups (food intake, body weight data, number of pups/litter, etc.). FISHER's EXACT test for pairwise comparison of each dose group with control group (mating and fertility indices, pup viability data). Two-sided WILCOXON-test (proportion of affected pups/litter with necropsy observations).

Test substance: 3-methyl-2-buten-1-al; degree of purity 97.7%

Conclusion: 3-methyl-2-buten-1-al was administered to Wistar rats of both sexes in drinking water at concentrations of 50, 200 and 800 ppm over a period of ca. 18 weeks in a one-generation
study. The corresponding mean uptake of TS was ca. 6, 21, and 77 mg/kg/d for both sexes, respectively.

Due to palatibility problems water intake was reduced in animals receiving 800 ppm solutions by ca. 20%. This suggests that no higher dose levels can be achieved by administration of TS via drinking water. Reduced water intake over the entire test period in both sexes and a transient decrease of food intake in males were the only signs of substance-related effects which were noted in the high dose groups only.

No change of any other parameter was noted, i.e. clinical, histopathological, and pathological findings were not different from the controls of either sex at any dose or at any time. This includes the histopathological examination of the reproductive organs of both sexes. Consequently no target organ could be identified.

The no observed adverse effect level (NOAEL) was 77 mg/kg/d for systemic toxicity for male and female F0 parental animals.

The NOEL for the F0 parental animals was 21 mg/kg/d in this study, based on the reduced water and food consumption at the top dose level.

Reliability: (1) valid without restriction
GLP guideline study. According to OECD 415. Provides valuable information for subchronic toxicity.

Flag: Critical study for SIDS endpoint

14-APR-2003 (54)

5.5 Genetic Toxicity 'in Vitro'

Type: other: SOS-Chromotest

System of testing: E. coli strain PQ37 or PM21
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: no data
Result: negative

Method: other
Year: 1990
GLP: no data
Test substance: other TS

Method: The SOS Chromotest using E. coli strain PQ37 was performed according to Quillardet and Hofnung, Mut. Res., 147, 65-78 (1985). The method using E. coli strain PM21 was described in Eder et al., Mutagenesis, 4, 179-186 (1989)
Result: SOSIP (SOS repair induction potency) was 4.8E-04. Maximal SOS induction factor was 1.7 (Reference table 2, page 102)
Test substance: 3-methyl-2-butenal; purity > 99.5%

Reliability: (4) not assignable
Documentation insufficient for assessment
14-APR-2003 (55)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration: 0, 4, 20, 100, 500, 2500 µg/plate
Cytotoxic Concentration: > 2500 µg/plate
Metabolic activation: with and without
Result: negative

Method: other
Year: 1979
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Reverse mutation according to Ames, B. N. et al., Proc. Nat. Acad. Sci. USA, 70, 2281-2285 (1973)
Result: 3-methyl-2-butenal did not increase the number of revertants in any of the test strains, neither in the absence nor in the presence of S9-mix.
No cytotoxicity was noted up to and including 2500 µg 3-methyl-2-butenal/plate.
The positive and negative controls yielded the expected results thus indicating valid test conditions.

Results for the positive controls:
(2-AA: 2-aminoanthracene, CCP: cyclophosphamide, MNNG: N-methyl-N'-nitro-N-nitrosoguanidine)

Strain	TA1535	TA100	TA1537	TA98	TA1538
	(fold increase, mean values)				
2-AA (+S9)	23.3	18.9	13.3	48.3	59.6
CCP (+S9)	9.0	2.1			
MNNG (-S9)	136.6	27.6	4.7	44.3	

Test condition: TEST SYSTEM

Base pair substitution (TA 1535, TA 100) and frameshift (TA1537, TA 1538, TA 98) tester strains with and without metabolic activation (+S9 or -S9, resp.).
ADMINISTRATION
Doses: 0, 4, 20, 100, 500, 2500 µg/plate tested with all tester strains both in the presence and in the absence of metabolic activation (remark: 2500 µg/plate was an accepted limit for the highest test concentration at that time).
Quaternary experiments.
Positive controls were included, 2-aminoanthracene and cyclophosphamide (+S9) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; -S9).
EVALUATION CRITERIA
Test substance is regarded positive if the control number of revertants is at least doubled and if reproducible results indicate a dose-response relationship.

Test substance: 3-methyl-2-butenal; substance S3 no. 79/192; purity > 98%
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restriction: E. coli WP2 or S. typhimurium TA 102 not included.
Flag: Critical study for SIDS endpoint
04-MAR-2004 (56)

Type: Ames test
System of testing: S. typhimurium TA 98, TA 100
Concentration: 20, 100, 500, 2000, 2500, 3000, 4000, 5000, 6000 µg/plate
Cytotoxic Concentration: 1000-3000 µg/plate
Metabolic activation: with and without
Result: positive

Method: other: similar to OECD 471
Year: 1991
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Reverse mutation according to Ames, B. N. et al., Proc. Nat. Acad. Sci. USA, 70, 2281-2285 (1973). Modification described by Rannug et al., Chem. Biol. Interaction, 12, 151-263 (1976) and Lutz et al., Mut. Res., 93, 305-315 (1982)
Result: Results without S9-mix
TA 100 was weakly positive (2.1-2.5-fold) in the 1st experiment at 2500-5000 µg/plate and more positive in subsequent experiments from 100-500 µg/plate (factor 1.7-4.6) with a maximum at 1000-3000 µg/plate (factor 6.0-8.1).
TA 98 showed a slight increase (factor 2.1) at 2500 µg/plate which was not concentration dependent.

Results with S9-mix
With TA 100 increased revertant numbers were seen at very high concentrations in the first experiment (1.6-fold at 5000 µg/plate) and in the second experiment (2.5-fold at 2000 µg/plate). In the third experiment mutagenicity was seen at lower concentrations (2.4-2.7-fold at 100-500 µg/plate).
TA 98 showed no increased number of revertants.

Cytotoxicity was observed only in the 2nd and 3rd experiment

using TA 100 from 1000-3000 µg/plate.

Complete solubility of the test substance in aqua dest. was given.

Test condition: TEST SYSTEM
Modified Ames preincubation test (similar to OECD 471), i.e. the Liquid suspension assay. Base pair substitution (TA 100) and frameshift (TA 98) tester strains with and without metabolic activation. Preincubation with test compound for 90 min at 37°C, followed by centrifugation and plating for 48 hrs.

ADMINISTRATION
Doses:
1st experiment 0, 20, 100, 500, 2500, 5000 µg/plate with TA 100 and TA 98.
2nd experiment 0, 2000, 3000, 4000, 5000, 6000 µg/plate with TA 100.
3rd experiment 0, 100, 500, 1000, 2000, 3000 µg/plate with TA 100.
Thrice experiments both in the presence and in the absence of metabolic activation. Positive controls were included:
2-aminoanthracene (+S9);
5-methyl-N'-nitro-N-nitrosoguanidine (MNNG; TA 100, -S9),
4-nitro-o-phenylendiamine (NPD, TA 98, -S9)

GLP
The study has formally no GLP status, however, the test was conducted and reported under GLP like conditions.

EVALUATION CRITERIA
Test substance is regarded positive if the control number of revertants is at least doubled and if reproducible results indicate a dose-response relationship.

Test substance: 3-methyl-2-butenal; substance S3 no. 88/363; purity 98%

Conclusion: Positive results of the study are ambiguous since the effects were not coherent between the three experiments and the dose-relationship was not clear.

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restriction: only 2 strains of S. typhimurium used.

Flag: Critical study for SIDS endpoint

04-MAR-2004 (57)

Type: Ames test
System of testing: S. typhimurium TA 100
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: no data

Method: other
Year: 1990
GLP: no data
Test substance: other TS

Method: Described in Eder et al, Toxicol Letters (1993). Two methods are described there:
(1) Ames test with a 30-min preincubation according to Maron&Ames both with and without metabolic activation
(2) Ames test with a 90-min preincubation and a 3-fold cell

density both with and without metabolic activation.
Remark: It was not specified which of the methods was used. It was not noted whether substance related, significant increases of the number of revertants were seen. It was stated in Table 1 that no SOS-chromotest had been performed with the Test Substance despite the fact that such data had been published by the same authors as early as 1990.
Result: In Table 1 it was reported that the number of revertants increased in TA 100 at a rate of 43 revertants/µmol 3-methyl-2-butenal.
Test substance: 3-methyl-2-butenal; no further information provided.
Reliability: (4) not assignable
Documentation insufficient for assessment
11-APR-2003 (58) (59)

Type: Ames test
System of testing: S. typhimurium TA 100
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: no data

Method: other
Year: 1990
GLP: no data
Test substance: other TS

Method: Ames test with a 30-min preincubation according to Maron&Ames both with and without metabolic activation
Result: It is reported (Table 2) that in TA 100 the number of revertants increases at a rate of 78 revertants/µmol 3-methyl-2-butenal, and that this effect was not significant.
Test substance: 3-methyl-2-butenal; purity > 99.5%
Reliability: (4) not assignable
Documentation insufficient for assessment
11-APR-2003 (55)

Type: other: SOS-Chromotest
System of testing: E. coli strain PQ37
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: no data
Result: ambiguous

Method: other
Year: 1993
GLP: no data
Test substance: other TS

Method: According to Quillardet and Hofnung, Mut. Res. 147, 5-78 (1985)
Result: SOSIP (SOS repair induction potency) was 5E-03. Maximal SOS

induction factor was 2.09. (Reference: Table IV on page 94)
Positive result according to the authors.
Test substance: 3-methyl-2-butenal; purity > 99.5%
Reliability: (4) not assignable
Documentation insufficient for assessment
14-APR-2003 (60)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: NMRI
Route of admin.: gavage
Exposure period: Single dose
Doses: 175, 350, 700 mg/kg bw
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 1991
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Stability of the test substance was verified by analytical data.
Range finding study: deaths were seen down to oral doses of 800 mg/kg bw. 700 mg/kg bw were survived by all animals but led to clear signs of toxicity such as irregular respiration,
apathy, piloerection, abdominal position within 15-30 minutes after dosing. Doses for the main study were selected based on these findings.
Main study:
Clinical signs: none were observed after vehicle only or after positive control substances. TS at 175 or 350 mg/kg bw: irregular respiration, piloerection and squatting posture were seen ca. 15 min after dosing. Some signs persisted for some hours. The same signs were seen after 700 mg/kg bw within 15 min. Squatting posture was still seen 1-6 hrs after dosing. Some signs persisted the next day. General state was poor.

Micronuclei counts at 24 hr after dosing, expressed as 0/00 in poly- (=pe) and normochromatic (=ne) erythrocytes:
Solvent control: pe=1.0 ne= 0.68
cyclophosphamide: pe=12.6 ne= 1.54
vincristine: pe=109.0 ne= 2.57
3-methyl-2-butenal 175 mg/kg bw: pe=1.4 ne= 1.18
3-methyl-2-butenal 350 mg/kg bw: pe=0.6 ne= 0.70
3-methyl-2-butenal 700 mg/kg bw: pe=1.7 ne= 0.92

At 16 hrs:
3-methyl-2-butenal 700 mg/kg bw: pe=1.0 ne= 1.0

At 48 hrs:
3-methyl-2-butenal 700 mg/kg bw: pe=1.4 ne= 0.8

Thus, no increase in normochromatic erythrocytes containing micronuclei was seen in any dose group at any sacrifice interval. No changes compared to negative controls were seen regarding small or large micronuclei in the three dose groups.
An inhibition of erythropoesis was seen with 700 mg/kg bw at all time intervals.

Test condition: TEST ORGANISM
70 NMRI mice, male and female, mean bw 28 g. 5 animals per dose, sex and time of bone marrow preparation were used for test substance and negative control, and 5 animals for each

of the positive control substances.
ADMINISTRATION
3-methyl-2-butenal was administered by gavage dissolved in water at a volume of 10 ml/kg bw. Concentrations were 175, 350, and 700 mg/kg bw. Negative controls received vehicle only.
Positive controls received the clastogen cyclophosphamide (20 mg/kg, in water) or the spindle poison vincristine (0.15 mg/kg) i.p. at 10 ml/kg bw. Femural bone marrow was prepared 24 hr post dosing, and at additionally at 16 hr and 48 hr after the high dose of 700 mg/kg bw.
EXAMINATION
Animals were observed for any clinical signs after dosing. Microscopic evaluation of ca. 1000 polychromatic erythrocytes from bone marrow preparations included the erythrocyte counts as detailed below under Evaluation Criteria.
EVALUATION CRITERIA
Generally, 1000 polychromatic erythrocytes per animal are evaluated for the parameters: (1) no. of polychromatic erythrocytes with/without micronuclei and calculation of clastogenic index (2) no. of normochromatic erythrocytes with/without micronuclei (3) calculation of the ratio polychromatic/normochromatic erythrocytes (4) no. of small(d<1/4) and large (d>1/4) micronuclei. No statistical data analysis performed.

Test substance: 3-methyl-2-butenal; substance S3 no. 90/734; purity 96.3%
Conclusion: Not clastogenic. No impairment of chromosome distribution in the course of mitosis.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
15-APR-2003 (61)

Type: Unscheduled DNA synthesis
Species: rat **Sex:** male
Strain: Wistar
Route of admin.: gavage
Exposure period: 3 and 14 hrs after single dose
Doses: 0, 350, 700 mg/kg bw
Result: negative

Method: OECD Guide-line 486
Year: 2001
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: TS stability in water at room temperature was verified.

Positive and negative control animals showed no signs of toxicity. 350 mg 3-methyl-2-butenal/kg bw were tolerated without any signs whereas 700 mg 3-methyl-2-butenal/kg bw led to strong clinical signs.

Cytotoxicity: cell viability and cell morphology were not affected by the treatment at any dose or time interval of hepatocyte harvesting.

DNA repair activity was clearly enhanced (positive) by 2-AAF after 3 and 14 hrs. Mean NNG was 5.84 (3 hrs) and 7 (at 14

hrs) with ca. 51% (58%) of the cells in repair (NNG >/= 5) at the early and the late time, respectively. In negative control cells 0% were in repair (NNG >/=5) at either time interval.
After 3-methyl-2-butenal treatment DNA repair activity was not enhanced compared with the negative control at either dose or time:
350 mg/kg bw: mean NNG count ca. - 4; 0% of cells in repair at both 3 and 14 hrs.
700 mg/kg bw: mean NNG count ca. - 4; 0% of cells in repair at both 3 and 14 hrs.

Therefore no statistical analysis was performed. The result was clearly negative.

Test condition: TEST ANIMALS
3 male animals were used in each of the 8 test groups. Meanbody weight was 254 g, the age range was 10-12 weeks. The
animals were singly housed.
ADMINISTRATION
3-methyl-2-butenal was diluted with water and administered by gavage (10 ml/kg bw) in doses of 350 and 700 mg/kg bw. Negative control animals received vehicle only.
Positive control animals received 50 mg 2-AAF (2-acetylaminofluorene) in corn oil.
EXAMINATIONS
Animals were observed for clinical signs of toxicity until sacrifice at 3 and 14 hrs after dosing. Hepatocytes were isolated by liver perfusion and viability was determined by the trypan blue exclusion method. Additionally cells were examined for morphological changes. 6 wells/animal with 400,000 cells each were subjected to the UDS assay procedure which included cell washing following to an attachment period, thymidine labeling, and autoradiography. Microscopical quantification of UDS was performed using an automatic image analyzer. 2 or 3 slides per test group were used. 25-50 cells per slide were randomly selected to achieve a total of 100 cells/animal. For each of the cells, nuclear and cytoplasmatic grains were counted (NG or CG, resp.). Calculations were made for mean NG and CG, absolute and mean net nuclear grains (NNG), and percentage of cells in repair for cells with NNG >0 and NNG >5.
EVALUATION
Acceptance criteria included comparison with negative and positive historical control data and liver cell viability of at least 70% from negative control animals. A substance was considered positive if a dose-related increase was demonstrated of both the mean NNG counts, and the percentage of cells in repair >/= 20 (NNG>/=5).

Test substance: 3-methyl-2-butenal; substance no. 00/0680-1; purity 97%

Conclusion: 3-methyl-2-butenal or its metabolites did not induce DNA damage as evidenced by the negative result in the UDS assay when given to rats in sublethal doses which led to clear signs of acute toxicity.

The test was valid as indicated by the results from negative and positive control animals. Moreover cell viability and cell morphology were not affected by the treatment.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
15-APR-2003 (62)

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

Type: One generation study
Species: rat
Sex: male/female
Strain: Wistar
Route of administration: drinking water
Frequency of treatment: 7 days per week
Premating Exposure Period
male: min 76 d
female: min 76 d
Duration of test: 18 wk
No. of generation studies: 1
Doses: 0, 50, 200, 800 mg/l; ca. 0, 6, 21, 77 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Parental: ca. 77 mg/kg bw
NOAEL F1 Offspring: ca. 77 mg/kg bw
NOEL parental : ca. 21 mg/kg bw
Result: Negative with respect to: systemic toxicity, reproductive performance and fertility, developmental toxicity up to the highest dose examined

Method: OECD Guide-line 415 "One-generation Reproduction Toxicity Study"
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Administration of the test substance in the drinking water was chosen for the following reasons: Application in the diet was not possible due to stability problems and long-term administration by inhalation or by gavage was considered as critical due to the corrosive properties.
Result: TS in drinking water
Stability and homogeneity was demonstrated.

F0 parental animals

TS intake: Calculated intake at dose levels 50, 200, and 800 ppm in drinking water was
F0 males: 4.9, 19.0, 72.0 mg/kg bw/d (premating)
F0 females: 6.4, 23.5, 81.8 mg/kg bw/d (premating)
F0 females: 6.2, 23.2, 82.0 mg/kg bw/d (gestation)
F0 females: 8.3, 31.9, 123.5 mg/kg bw/d (lactation)

The mean dose administered for both sexes during premating was ca. 5.7 mg/kg bw/d, ca. 21.3 mg/kg bw/d, and ca. 76.9 mg/kg bw/d in the low, mid and high dose groups, respectively.

Mortality
One high dose female was found dead during premating wk 10. Erosions/ulceration of glandular stomach mucosa was found at

necropsy, but this was assessed as being not causative for death. No other mortalities occurred.

CLINICAL OBSERVATIONS
Clinical findings: No substance-related signs or any disturbance of behavior noted in all male and female F0 animals during the entire administration period.
Water consumption: Unchanged at the low and intermediate dose level, significantly reduced during premating (-20% males, -24% females) and in females during gestation (-17%) and lactation (-10%). This was regarded as a substance-related effect, probably due to bad taste and/or smell of TS solutions.
Food consumption: Significantly reduced in high dose males during the first weeks of treatment (wk 0-4), and similar to control animals thereafter. No other relevant changes noted.

Body weight, body weight gain: Not influenced by TS in any group at any time interval.

Male reproduction data: Male mating index was 96% in the test group at 50 ppm and 100% in all other test groups. Male fertility index was 96% in all groups.
Female reproduction data: Female mating index was 96% in the test group at 50 ppm and 100% in all other groups. Fertility index was 100% in the test group at 50 ppm and 96% in all other test groups. Duration of gestation was similar in all groups (21.8-22.0 days). Gestation index was 100% for all groups.
Live birth index varied between 96 and 100%. It was significantly lowered at 800 ppm due to a higher number of stillborn pups (10 vs 3 in the control). This was not regarded as being substance-related for the following reasons:
1. The increased number of stillborn pups was due to 2 dams which had 4 stillborn pups each.
2. Viability and lactation indices as indicators of pup mortality were not affected at 800 ppm
3. The values reflect the range of biological variation in the strain of rats used.
Pathology:
Gross lesions were noted in glandular stomach (erosion/ulcer), cecum, liver, testes (size reduced) epididymides (size reduced) oviducts (cyst), uterus (cyst), eyes and skin. However, these were single observations or distributed over control and treated groups without evidence of relationship to treatment. The same applies to histopathology.

2. F1 generation
The mean number of delivered pups per dam were not affected. The viability indices were 97/98/99/99% in the control to high dose group, respectively. The lactation indices reached 99/100/99/100% in the respective groups (0/50/200/800 ppm). Thus, there was no differences between treated and control groups. Sex ratio were unchanged. Pup body weight and body weight gain of male and female pups were similar to control pups and not affected by treatment.
No clinical signs were noted in F1 pups up to weaning. Necropsy of pups (stillborn; pups that died intercurrently;

culled pups; terminal examination) did not reveal specific lesions or increased incidences or a target organ that could be related to treatment.

Overall, no substance-related adverse effects were noted in F0 parental animals and F1 pups in groups at 50 and 200 ppm. Changes noted in F0 animals at 800 ppm were a decreased water intake in both sexes and a transient decrease of food intake of males during the first 4 weeks of treatment. There were no other differences in the high dose group F0 and F1 animals compared to controls.

Test condition: TEST ANIMALS
25 male and female Wistar rats (CrlGlxBrlHan:Wi) were used per dose level. At the beginning of treatment, animals were 34+/- 1 days old. Mean body weights were 107 (95-117)g for male and 96 (86-105)g for female animals. During the study period the animals were housed singly.
OVERALL EXPERIMENTAL DESIGN
After acclimatization F0 animals were treated until day 21 after parturition of their progeny. Animals were mated 76 after beginning of treatment and allowed to rear their F1 pups until day 21 after parturition. Then all F0 and F1 animals were sacrificed and examined.
Litters were standardized on day 4 after parturition thus that each litter consisted of 4 male and 4 female F1 pups, where possible.
EXPOSURE/ADMINISTRATION
Animals received TS in the drinking water at concentrations of 0, 50, 200, and 800 ppm.
MATING
Animals from the same dose groups were mated 1:1 after at least 76 d after beginning of treatment.
EXAMINATIONS
TS stability for 4 days was examined. Homogeneity was given by complete water solubility. Concentration control analyses were performed at start, middle and end of the treatment period.
Parental animals were observed daily during the study. Fetuses were removed from the uterus and examined as described below.
Observations and examinations included
1. Parent animals: Daily check for signs of toxicity, mortality. Water consumption was determined once a week during premating for 3-d periods, and for females on days 0-1, 6-7, 13-14, and 19-20 post coitum during gestation, and on days 1-2, 4-5, 7-8, and 14-15 after parturition. No water consumption was determined on days 20-21 as required in OECD 415 since F1 pups consume considerable amounts of water during this time. Also, no water consumption was determined for females without sperm or litters, and for males after 10th study week. Food consumption was determined weekly. After 10th week in pregnant females weekly during gestation, days 1-4, 4-7, and 7-14 during lactation. No determination days 14-21 as required by OECD 415 since pups start to consume food. Body weight were determined weekly and in females with litters on days 4, 7, 14, and 21 during lactation.
Male and female reproduction data (mating index and fertility index per sex) were calculated.
Terminal examination of F0 parental animals included gross

pathology and histopathology of organs of the reproductive system as required by OECD 415.
2. Litters/pups: Number and viability of pups was determined as soon as possible. Viability indices were calculated at days 4 and 21 after parturition. Sex was determined on the day of birth by observing the anogenital distance and finally confirmed at necropsy. Pup body weights were determined on days 1, 4, 7, 14, and 21 after birth. Clinical symptoms were examined daily. Terminal examination after sacrifice on day 21 after parturition included external examination and macroscopical assessment of internal organs.

STATISTICAL DATA EVALUATIONS
DUNNETT's test was used to compare treated and control groups (food intake, body weight data, number of pups/litter, etc.). FISHER's EXACT test for pairwise comparison of each dose group with control group (mating and fertility indices, pup viability data). Two-sided WILCOXON-test (proportion of affected pups/litter with necropsy observations).

Test substance: 3-methyl-2-buten-1-al; degree of purity 97.7%

Conclusion: 3-methyl-2-buten-1-al was administered to Wistar rats of both sexes in drinking water at concentrations of 50, 200 and 800 ppm over a period ca. 18 weeks in a one-generation study. The corresponding mean uptake of TS was ca. 6, 21, and 77 mg/kg bw/d for both sexes, respectively.

Due to palatibility problems water intake was reduced in animals receiving 800 ppm solutions by ca. 20%. This suggests that no higher dose levels can be achieved by administration of TS via drinking water.

Reduced water intake over the entire test period in both sexes and a transient decrease of food intake in males were the only signs of substance-related effects which were noted in the high dose groups only.

No change of any other parameter was noted, i.e. clinical and pathological findings, systemic toxicity, reproductive performance and fertility, developmental toxicity were not different from the controls of either sex at any dose or at any time. This includes the histopathological examination of the reproductive organs of both sexes.

Therefore, the no observed adverse effect level (NOAEL) is 77 mg/kg/d for reproductive performance and fertility, systemic toxicity, and developmental toxicity for F0 parental animals and their F1 progeny.

The no-effect-level (NOEL) for the F0 parental animals was 21 mg/kg/d in this study based on the reduced water and food consumption at the
top dose level.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

04-MAR-2004 (54)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Exposure period: day 6 to 19 post coitum, gestation
Frequency of treatment: 7 administrations per week
Duration of test: 21 days
Doses: 0, 50, 150, 300, 450 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 300 mg/kg bw
NOAEL Teratogenicity: = 300 mg/kg bw
NOAEL Fetotoxicity : = 300 mg/kg bw
NOAEL Embryotoxicity : = 300 mg/kg bw
Result: negative at the maximum applicable dose of 300 mg/kg bw/d

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2001
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Studies were carried out according to or exceeding the requirements of the guidelines OECD 414 (2001), EC 88/302, B 31(1988), US EPA OPPTS 870.3700
Remark: Since none of the high dose dams (450 mg/kg bw/d) survived until sacrifice due to unexpectedly high toxicity a second study (study report no. 30R080/00125) with only two test groups (0 and 300 mg/kg bw/d) was conducted. The combined results from these two complementary studies are described in this summary.
Result: TS ANALYSES

Stability and homogeneity were demonstrated. Concentrations ranged between 84-98.6% of nominal concentrations. The lower value was found at the lowest dose level at beginning of the study only and has no implications for the overall assessment of the study.

MATERNAL EFFECTS
Only pregnant dams or pregnant dams sacrificed at schedule were used for calculations. Animals which were totally or partially excluded were as follows: in the 1st study one animal each from control, low and intermediate dose level; all high dose animals. In the 2nd study one animal at 300 mg/kg bw/d was not pregnant.

MORTALITY
Of the group at 450 mg/kg bw/d five animals were found dead between d 8 to 10 p.c., and all others were sacrificed due to poor condition at the same interval. There were no mortalities in the other test groups.

CLINICAL DATA
CLINICAL SYMPTOMS
High dose, 450 mg/kg bw/d: most prominent findings were abdominal position (16/25), transient salivation in almost all animals, lacrimation (11/25) shortly after treatment, apathy (6/25), piloerection (15/25). Distinct reduction in food consumption and body weight. Necropsy revealed

ulcerations/erosions of stomach mucusa and/or mucoid enteritis.
High intermediate dose, 300 mg/kg bw/d: from day 6 p.c. onwards transient salivation was noted in 23/25 rats shortly after treatment and persisted for a short period of 1-2 hrs. No salivation was noted after cessation of treatment. No other clinical signs noted.
Low intermediate dose, 150 mg/kg bw/d: on days 15-19 p.c., i.e. towards the end of the treatment period, transient salivation was noted in 6/25 rats shortly after treatment; it persisted for a short period of 1-2 hrs. Piloerection was noted in one rat which also showed vaginal hemorrhage and almost no food consumption, associated with body weight loss.
Low dose, 50 mg/kg bw/d: no other observation than piloerection in one animal noted.

No salivation was noted after cessation of treatment. The effect was regarded as being substance-related due to bad taste, not as an adverse effect.

FOOD INTAKE; BODY WEIGHTS
Food intake was drastically reduced (-36%) in high dose group animals at 450 mg/kg bw/d during day 8-10 p.c.; consequently body weight was also significantly lowered. Mean food intake of animal groups receiving 50, 150, and 300 mg/kg bw/d was similar to or even exceeded that of control animals.
Body weight and body weight gain of treated animals (except those at 450 mg/kg bw/d) was similar to or slightly higher than that of control groups. Also, corrected body weight gain was comparable in all dose groups.

TERMINAL EXAMINATION OF DAMS
Mean gravid uterus weight was not influenced by treatment in groups at 50, 150, and 300 mg/kg bw/d.

Necropsy findings: in high dose animals (450 mg/kg bw/d) gastrointestinal tract findings included erosions of stomach mucosa, reddening of intestines, gas or fluid in stomach or intestines. In animals at 300 mg/kg bw/d, dilated renal pelvis (2/25) and reddened intestines (1/25) were noted. Both of these findings were considered to be random in nature due to their scattered occurrence. There were no findings in animals at 50 or 150 mg/kg bw/d.

REPRODUCTION DATA
There were no significant or biologically relevant differences between control animals and animals at 50, 150, and 300 mg/kg bw/d with respect to conception rate, mean number of corpora lutea and implantation sites, pre- or postimplantation loss, number of resorptions, and viable fetuses. In each of the two studies one control animal had only early resorptions but no viable fetuses. However, all differences were within the normal range and within historical control data.

EXAMINATION OF FETUSES
(Note: no fetuses obtained at 450 mg/kg bw/d)
Sex distribution was unchanged in all treatment groups.

Placental weight was unchanged in all treatment groups. Mean fetal body weight was not influenced in all treatment groups.

External malformations:
Group at 300 mg/kg bw/d: 1/232 control fetuses (=0.4%) in 1/24 litters (=4.2%); mean affected fetuses/litter =0.5%. 0/226 test group fetuses (24 litters); mean affected fetuses/litter =0.0%.
Group at 150 mg/kg bw/d: 0/221 control fetuses in 23 litters;
mean affected fetuses/litter =0.0%. 1/225 (=0.4%) test group fetuses (24 litters; = 4.2%); mean affected fetuses/litter =1.0%.
Group at 50 mg/kg bw/d: 1/228 test group fetuses (24 litters;
=4.2%); mean affected fetuses/litter =0.4%.
There were no external variations or so called unclassified observations.

Soft tissue examination:
Malformations: Only one soft tissue malformation was observed in the mid group. The mean percentages of affected fetuses per litter with soft tissue malformations amounted to 0, 0, 2.1 and 0 % for the respective test groups

Soft tissue variations: In both the 1st and the 2nd study two soft tissue variations (dilatation of renal pelvis and/or ureter) were noted in each group including control. Mean percentages of affected fetuses/litter were 9.7% (control 1), 17.4% (50 mg/kg bw/d), 10.8% (150 mg/kg bw/d), 8.0% (control 2), 9.0% (300 mg/kg bw/d).

Skeletal malformations:
Malformations of the fetal skeleton were observed at low incidences in single fetuses of the control and dosed groups. The noted skeletal malformations appeared without a relation to dosing, without biologically relevant differences between the groups and/or can be find at a comparable frequency in the historical control data.
Incidences of malformations were as follows:
Group at 300 mg/kg bw/d: 12/123 control fetuses (=1.6%) in 2/24 liters (=8.3%); mean affected fetuses/litter =1.9%. 3/120 test group fetuses (=2.5%) in 3/24 litters (13%); mean affected fetuses/litter =2.4%.
Group at 150 mg/kg bw/d: 1/117 control fetuses (0.9%) in 1/23
liters (4.3%); mean affected fetuses/litter =0.9%. 5/116 (=4.3%) test group fetuses (1/24 litters; = 4.2%); mean affected fetuses/litter =4.2%.
Group at 50 mg/kg bw/d: 5/119 test group fetuses (5/24 litters; =21%); mean affected fetuses/litter =4.3%.
These data include all stunted fetuses from one mid dose animal which should be excluded from the overall assessment since this dam showed massive impairment of general condition.

Skeletal variations:
The number of affected fetuses/litter was significantly increased for incomplete ossification of basisphenoid, and

sternebra (with unchanged cartilage), supernumeral thoracic vertebra, and extra 14th rib in test groups receiving 50 or 150 mg/kg bw/d, but the incidences were well in the range of historical controls, and no dose-dependency was noted. The same applies to the group at 300 mg/kg bw/d. The mean percentage of affected fetuses per litter with skeletal variations amounted to 95.0, 94.0, 89.5 and 89.5 and 85.5% (0, 50, 150, mg/kg bw/d and 0, 300 mg/kg bw/d).

Overall, the examination of fetal external, soft tissue and skeletal malformations and variations revealed no difference between treated and control groups since incidences were not affected or inside historical control range and of no biological relevances where statistical significance was given. Moreover no dose-dependency was given, and no specific lesion or target organ was identified. Therefore administration of up to 300 mg/kg bw/d of 3-methyl-2-buten-1-al to pregnant rats did not cause prenatal developmental toxicity and no teratogenicity.

Test condition: TEST ORGANISM
25 time-mated female rats per dose, age ca. 70-84 d at beginning of the study (day 0 post coitum, p.c.; detection of sperm), were used. Body weights ranged between 143 to 186 g on day 0 p.c.. Animals were housed singly from day 0-20 p.c.
ADMINISTRATION
TS was administered by oral gavage once a day from implantation to one day prior to expected parturition, i.e. d 6-19 p.c. TS suspended in 0.5% CMC in doubly distilled water was given at 0, 50, 150 and 450 mg/kg bw/d in the initial study and at 0 and 300 mg/kg bw/d in the second, complementary study.
EXAMINATIONS
TS: analysis of TS purity and stability in water for at least 4 days was performed; analysis of TS solutions using GC before study started and after it ended was performed at various dose levels (50, 300, 450 mg/kg bw/d) in triplicate in order to verify concentrations and homogeneity.
Dams:
The animals were examined at least daily for clinical symptoms. Mortality was checked twice a day or once on Saturday/Sunday. Food and water consumption, body weight on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c.. Corrected body weight gain was calculated after terminal sacrifice as [terminal bw on d 20- (uterus weight+bw d 6 p.c.)].
Examinations at termination:
Dams: Sacrificed on day 20 pc. Necropsy included gross pathology assessment. Uterus and ovaries were removed with subsequent examination and recording of: unopened uterus weight; no. of corpora lutea; no. and classification of implantation sites as live fetuses or dead implantations (early, late resorptions, dead fetuses). Calculation of conception rate and pre- and postimplantation losses.
Fetuses: were weighed and sexed by anogenital distance observation (which was later confirmed by internal examination of those fetuses which were preserved in BOUIN's solution), and macroscopically examined (viability, condition of placenta, fetal membranes, umbilical cords, placental weight). Approx. 50% of all fetuses were subjected

to soft tissue examinations after fixation in BOUIN's solution, the other 50% of fetuses was examined for skeletal changes following ethyl alcohol fixation.
EVALUATION
In order to minimize bias, dams were sacrificed in a randomized order, and all examinations subsequent to weighing the unopened uterus were conducted without knowledge of the treatment group. The glossary of Wise et al. (1997) and the definitions proposed by Chahoud et al. (1999) were used during assessing fetal findings. Thus, "malformation" was used for a permanent structural change that is likely to adversely affect the survival or the health; "variation" is used for a change that also occurs in fetuses of control animals and is unlikely to adversely affect survival or health. This includes delays in growth or morphogenesis that has otherwise followed a normal pattern of development. "Unclassified observation" or "unclassified cartilage observation" were used if fetal findings could not be classified as malformations or variations.
STATISTICAL EVALUATION of data included FISHER's Exact Test for conception rate, mortality of the dams, and number of litters with fetal findings; WILCOXON Test for proportions of fetuses with malformations and/or variations in each litter; and DUNNETT's Test for all other data including water consumption and body weight gain.
HISTORICAL CONTROL DATA
The historical control data used for interpretation of findings refer to the same test facility, the same rat strain and supplier of the animals and cover a period of about 5 months (July 2001 - November 2001, 6 studies).

Test substance: 3-methyl-2-buten-1-al; degree of purity 97.7%

Conclusion: Maternal toxicity was noted exclusively after oral administration of 450 mg/kg bw/d to pregnant rats. All animals died or were moribund after few days of treatment. Transient salivation was noted in animals receiving 300 and - less pronounced- 150 mg/kg bw/d. Since this was regarded as a palatibility problem rather and not as an adverse effect, no signs of maternal toxicity were noted at 300 mg/kg bw/d or below.

At doses up to and including 300 mg/kg bw/d reproduction data of dams were unchanged, i.e. pre- and postimplantation changes were unchanged as was the proportion of viable fetuses. This indicates that the TS was not embryo- or fetotoxic at these dose levels.

Fetal weight and sex distribution was unchanged up to and including 300 mg/kg bw/d. Further, external examination of fetuses did not indicate impaired development since observed delays in ossification were increased compared with the concurrent control groups but well in the range of historical data.

Administration of TS up to and including 300 mg/kg bw/d evoked no signs of teratogenicity, since no specific lesions or target organs were identified. Findings were not different from those frequently noted in controls at incidences well within the range of historical control data. Moreover, scattered findings revealed no dose-relation.

Based on these findings the no observed adverse effect level (NOAEL) for maternal toxicity and for prenatal developmental toxicity is 300 mg/kg bw/d.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

09-JUL-2003 (63) (64)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: no data available

24-JUL-2002

5.11 Additional Remarks

Type: Biochemical or cellular interactions

Method: Adduct isolation and characterization after in vitro incubation of enals with nucleosides and 5'-mononucleotides

Result: 3-methyl-2-butenal (and also pentenal and hexenal) formed adducts with deoxyguanosine and 2'-deoxyguanosine-5'-monophosphate, but not with any other nucleosides or nucleotides. Adduct formation led to two different adducts, i.e. 1,N2-cyclic adduct (I) and to a lesser extent to 7,8-cyclic adducts (II) with all enals. A general tendency of decreasing adduct formation was seen with increasing degree of substitution, possibly due to steric hindrance.
Basically similar adducts were formed as with crotonaldehyde. Since DNA adducts are often considered as premutagenic lesions the authors suggest to consider the enals (including 3-methl-2-butenal) to be carcinogenic like crotonaldehyde.

Test condition: Beta-alkyl-substituted acroleins (3-methyl-2-butenal; additionally 2-pentenal, 2-hexenal and 2,4-hexadienal) were stirred at 90°C for 4-6 days. After hydrolyis, modified nucleobases were separated on a LH 20 column and subjected to FT-IR-UV spectroscopy, mass spectrometry, NMR spectroscopy.

Conclusion: In vitro, 3-methl-2-butenal forms adducts with deoxyguanosine and its 5'monophosphate, but not with other nucleosides or nucleotides.

Reliability: (4) not assignable

04-MAR-2004 (65)

Type: Biochemical or cellular interactions

Method: Considerations on structur-activity relationship, linking information from adduct characterization after in vitro incubation of a,b-unsaturated carbonyl compounds with deoxyguanosine and deoxyguanosine-5'-mononucleotide with results from Ames test.

Result: 3-methyl-2-butenal was among the many a,b-unsaturated carbonyl compounds that formed type I adducts, i.e. a saturated 1,N2-cyclic adduct.

In bacterial test systems, 3-methyl-2-butenal failed to induce the number of revertants in the preincubation Ames test using TA 100, and it was borderline positive in the SOS chromotest using E. coli.

Conclusion: According to the authors, a,b-unsaturated carbonyl compounds forming saturated cyclic deoxyguanosine adducts are mutagenic in TA 100, and to a lesser degree also in TA 1535.

3-methyl-2-butenal which formed cyclic adducts. However, no mutagenicity was seen with TA 100 in the Ames test, and results of the SOS chromotest using E. coli was borderline positive. Thus the proposed relation was not convincing in the case of 3-methyl-2-butenal.

Reliability: (4) not assignable

04-MAR-2004 (55)

Type: Biochemical or cellular interactions

Method: Attempt to combine chemical reactivity in vitro (adduct formation) with biological effects (Ames test using TA 100; SOS chromotest using E. coli; alkaline elution for DNA strand break detection using 1210 mouse leukemia cells) for several a,b-unsaturated carbonyl compounds.

Result: Reportedly, 3-methyl-2-butenal significantly induced the SOS DNA repair system (induction factor=2) in the SOS chromotest with a SOS induction potency (SOSIP) of 5E-03. SOSIP was expressed as 1/nmol. No concentration was given for 3-methyl-2-butenal.

Conclusion: The paper covers several a,b-unsaturated carbonyl compounds but provides very little information on 3-methyl-2-butenal.

Reliability: (4) not assignable

04-MAR-2004 (60)

Type: Biochemical or cellular interactions

Method: Proposal of a screening for mutagenicity and carcinogenicity based on structure-activity relationships

Remark: No data provided pertaining to: number of revertants in control ; 3-methyl-2-butenal concentrations tested; number of revertants at these doses; number of replicates

Result: 43 revertants/µmol 3-methyl-2-butenal in the Ames test using TA 100.

Conclusion: Data cited from earlier work do not provide strong evidence for the structure-activity relation proposed by the authors.

Reliability: (4) not assignable

18-APR-2002 (59)

Type: other: Chemical substance review including toxicological data

Result: The chemical substance report summarizes available information pertaining to chemico-physical properties, production volumes (ca. 5,000 to per annum at that time; plans of future expansion were mentioned, i.e. to ca. 10,000

to per from 1999 onwards), and use. The vast majority (ca. 99%) of 3-methyl-2-butenal serves as intermediate and is further processed to citral or vitamins etc. About 1% of the production volume is sold and little is known about how this 1% is processed.

Environmental and toxicological data are provided along with estimates of human exposure.

Conclusion: No further environmental studies were deemed necessary. Studies pertaining to reproduction toxicity and carcinogenicity were not regarded as being urgent due to the facts that
- the vast majority of 3-methyl-2-butenal is used as industrial intermediate
- which leads to a small potential of exposure, and
- the absence of genotoxic properties in-vivo.

Reliability: (1) valid without restriction
Meets national standard methods (BUA-Existing chemicals assessment)

14-OCT-2002 (66)

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing

Fire/Exp. Prot.: Ensure thorough ventilation of stores and work areas. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Storage Req.: Keep tightly closed in a dry, cool and well-ventilated place. Protect from air.

Remark: Personal precautions: respiratory protection

Environmental precautions: Do not let product enter drains.

Methods for cleaning up: Large spillages should be dammed-off and pumped into containers; soak up remainder with absorbent material and dispose of in accordance with local regulations.

Transport information

Land transport

ADR/RID	Class: 8	Packaging group: II
Warning panel	Hazard-no: 83	Substance no.: 2920

UN-No: 2920
Description of the goods: AETZENDER FLUESSIGER STOFF, ENTZUENDBAR, N.A.G. (3-METHYLBUTEN-2-AL-1).

Inland waterway transport

ADN/ADNR	Class: 8	Packaging group: II

Description of the goods: AETZENDER FLUESSIGER STOFF, ENTZUENDBAR, N.A.G. (3-METHYLBUTEN-2-AL-1).

Sea transport

IMDG/GGVSee	Class: 8	UN-No: 2920 PG: II
	EMS: 8-15	MFAG: 300

Marine pollutant: no
Proper technical name: CORROSIVE LIQUID, FLAMMABLE, N.O.S. (3-METHYLBUTEN-2-AL-1).

Air transport

ICAO/IATA	Class: 8	UN/ID-No.: 2920 PG: II

Proper technical name: CORROSIVE LIQUID, FLAMMABLE, N.O.S. (3-METHYLBUTEN-2-AL-1

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.2 Fire Guidance

Prot. Equipment: Wear self-contained breathing apparatus and protective suit.
Ext. Medium: carbon dioxide (CO2), dry extinguishing media, foam, water
Add. Information: Dispose of fire debris and contaminated extinguishing water in accordance with local regulations.

Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

8.3 Emergency Measures

Type: other: general advice

Remark: Immediately remove contaminated clothing. If danger of loss of consciousness, place patient in recovery position and transport accordingly. Apply artificial respiration if necessary.
First-aiders should pay attention to their own safety.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: injury to persons (skin)

Remark: Immediately wash thoroughly with plenty of water, apply sterile dressings, consult a skin specialist.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: injury to persons (eye)

Remark: Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: injury to persons (oral)

Remark: Immediately rinse mouth and then drink plenty of water, do not induce vomiting, summon physician.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: injury to persons (inhalation)

Remark: immediately inhale corticosteroid dose aerosol (e.g. dexamethazone); keep patient calm, remove to fresh air, summon medical help.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: other: note to physician

Remark: Treat according to symptoms (decontamination, vital functions), no known specific antidote, administer corticosteroid dose aerosol to prevent pulmonary odema (e.g. dexamethazone).
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

Type: accidental spillage

Remark: Large spillages should be dammed-off and pumped into containers; soak up remainder with absorbent material and dispose of in accordance with local regulations.
Flag: non confidential, Critical study for SIDS endpoint
04-NOV-2002 (1)

8.4 Possib. of Rendering Subst. Harmless

8.5 Waste Management

Memo: other: Product must be disposed of by special means, e.g. suitable incineration, in accordance with local regulations.

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.6 Side-effects Detection

8.7 Substance Registered as Dangerous for Ground Water

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