FOREWORD INTRODUCTION

ISOBUTYL ISOBUTYRATE

CAS N°: 97-85-8

SIDS Initial Assessment Report

For

SIAM 20

Paris, France, 19-22 April 2005

1. Chemical Name:	**Isobutyl isobutyrate**
2. CAS Number:	97-85-8
3. Sponsor Country:	United States National SIDS Contact Point in Sponsor Country: Oscar Hernandez, Director U.S. Environmental Protection Agency Risk Assessment Division (7403 M) 1200 Pennsylvania Avenue, NW Washington DC 20460 Phone: (202) 564-7461
4. Shared Partnership with:	American Chemistry Council, Oxo Process Panel
5. Roles/Responsibilities of the Partners:	Not applicable
• Name of industry sponsor /consortium	American Chemistry Council Barbara Francis, Oxo Process Panel 1300 Wilson Blvd Arlington, VA 22209 Phone: (703) 741-5609
• Process used	Robust Summaries/dossiers, the SIAR, and the SIAP were drafted by the Oxo Process Panel's toxicologists. Documents were reviewed by the Oxo Process Panel and the United States Environmental Protection Agency.
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme?	The American Chemistry Council's Oxo Process Panel submitted a test plan and robust summaries for this chemical to the U.S. Environmental Protection Agency in December 2001, under the International Council of Chemical Associations (ICCA) Global Initiative on High Production Volume (HPV) Chemicals Program.
7. Review Process Prior to the SIAM:	Members of the Oxo Process Panel conducted a comprehensive literature search (ChemiIDplus, Hazardous Substances Database (HSDB), RTECS, TOXLINE, DOSE, Fisher Scientific MSDS, Scifinder Registry and Chemlist, Chemical Abstracts, and CHEMFATE) on March 10, 2000. In addition,

	the IUCLID database was searched for references, as was the internal company database of the only US manufacturer; Eastman Chemical Company. Documents were prepared by the Panel and reviewed by industry toxicologists prior to submission to the United States Environmental Protection Agency (U.S. EPA). The EPA conducted reviews of submitted data and offered comments to industry. The EPA submitted documents to OECD for consideration at SIAM 20.
8. Quality check process:	The quality of existing data was determined using guidance provided in the Manual for Investigation of HPV Chemicals, Chapter 3: Data Evaluation (OECD, 2002).
9. Date of Submission:	22 December 2003
10. Date of last Update:	8 September 2005
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	97-85-8
Chemical Name	Isobutyl isobutyrate
Structural Formula	CH_3-CH(CH_3)-CH_2-O-C(=O)-CH(CH_3)-CH_3

SUMMARY CONCLUSIONS OF THE SIAR

Analogue justification

Data from isobutanol toxicity studies have been included in the human health section. Data from isobutanol are useful when assessing the hazards associated with the systemic toxicity of isobutyl isobutyrate exposure due to the rapid and complete metabolism of isobutyl isobutyrate to isobutanol and isobutyric acid in vivo. Isobutanol is then further metabolized to isobutyric acid. Therefore, exposure to isobutyl isobutyrate via dermal, inhalation, and water or dietary administration is expected to result in the rapid appearance of isobutanol and isobutyric acid in the systemic circulation. Since exposure to either isobutyl isobutyrate or isobutanol results in systemic exposure to isobutanol and isobutyric acid, systemic toxicity data from studies that administer isobutanol directly are useful in identifying hazards associated with isobutyl isobutyrate exposure. Data from studies conducted with isobutyric acid were not included, since there were none available. The toxicokinetics of the metabolic reaction is documented and explained below.

The acute aquatic toxicity database of isobutyl isobutyrate was supported using data from a structural analog, compound, 2-ethylhexyl acetate (CAS# 103-09-3), alleviating the need for additional testing on isobutyl isobutyrate. Data from structurally similar compounds may be used to address the aquatic toxicity of isobutyl isobutyrate.

Human Health

Metabolism/toxicokinetic studies have been conducted with isobutyl isobutyrate using intravenous injections. Isobutyl isobutyrate levels peaked immediately after injection and rapidly decreased thereafter. The calculated T1/2 by one-compartment modeling was 11.1 seconds. Isobutyl isobutyrate is metabolized extremely rapidly in vivo to isobutanol and isobutyric acid. Isobutanol is then further oxidized to isobutyric acid.

The oral LD_{50} in rats is >6400 mg/kg bw. Dermal LD_{50} in male rabbits was >10 ml/kg bw. Inhalation LC_{66} values for vapor exposures were 5423 ppm (31.94 mg/L) in rats (6 hours of exposure). Exposures to 658 ppm caused no deaths in 6 hours. Isobutyl isobutyrate is a slight skin irritant. Data for eye irritation and skin sensitisation are not available.

An 18-week oral gavage study in rats at dose levels of 0,10,100, and 1,000 mg/kg/bw/day with isobutyl isobutyrate reported an increase in relative spleen weights following a slight decrease in terminal body weights in the male animals treated with 1,000 mg/kg/bw/day. The lack of histopathological findings in the spleen and the lack of effect in the female animals resulted in the NOAEL being 1,000 mg/kg/bw/day. Studies with isobutanol generally corroborate this value although acute signs of toxicity were noted immediately after oral dosing with isobutanol. The use of different vehicles (corn oil with isobutyl isobutyrate and distilled water with isobutanol) affects the rate of absorption of these related materials and explains the presence or absence of clinical signs immediately after oral exposures. An *in vitro* mutagenicity study in bacteria indicates that isobutyl isobutyrate is not a genotoxicant. In addition, isobutanol was negative in an *in vivo* mouse micronucleus study. An inhalation two-generation reproductive toxicity study conducted with Isobutanol (up to 2500 ppm; 7.58 mg/L) did not cause any parental systemic, reproductive, or neonatal toxicity when administered for two generations via whole-body exposure. No adverse developmental effects were noted in rats or rabbits exposed up to 10mg/L Isobutanol during gestation.

Environment

The available physicochemical data are adequate to describe the properties of isobutyl isobutyrate. Isobutyl isobutyrate has a melting point of -80.7 °C, boiling point of 148.6 °C and vapor pressure of 5.8 hPa at 25^{0}C, a water solubility of 520 mg/L at 20^{0}C and a calculated log K_{ow} of 2.68. The photochemical removal of isobutyl isobutyrate as mediated by hydroxyl radicals occurs with a calculated half-life of 1.947 days. Isobutyl isobutyrate is readily biodegradable under aerobic conditions, based on data for isopropyl- and isobutyl-acetate. Isobutyl isobutyrate volatilizes easily from moving rivers, but volatilizes only moderately from quiescent lakes and other surface water bodies (calculated volatilization half-lives of 1.67 hours from a river and 4.955days from a lake). Isobutyl isobutyrate is not persistent in the environment and is not likely to bioaccumulate in food webs. Using a calculated log K_{ow} of 2.68, the BCF is 23.1. Based on Level III distribution modeling (assuming equal releases to water, air, and soil) it is estimated that the majority of isobutyl isobutyrate released to the environment will partition into water (34.4%) and soil (52.7%), with a smaller amount in air (12.6%). The stability of isobutyl isobutyrate in water is pH dependent, at neutral pHs (7) the $T_{1/2}$ = 9.2 years at 25^{0}C and at higher pHs (8) the $T_{1/2}$ is shortened to 337 days.

Except for a study with the aquatic invertebrate, *Daphnia magna*, aquatic toxicity data are not available for isobutyl isobutyrate. Data for the structurally similar 2-ethylhexyl acetate (CAS# 103-09-3) were used to supplement the data for isobutyl isobutyrate. For fish, two studies with rainbow trout (*Oncorhynchus mykiss*) and 2-ethylhexyl acetate are available. Acute 96-h LC_{50}s of 8.27 and >4.2 mg/L were reported for 2-ethylhexyl acetate, respectively. Data are available with isobutyl isobutyrate and the invertebrate *Daphnia magna* with 48-h EC50 values of 55.8 to 59.3 mg/L reported. In addition, a daphnid study with 2-ethylhexyl acetate reported a 48-h EC_{50} of 22.9 mg/L. Data are available with 2-ethylhexyl acetate and the green alga *Selenastrum capricornutum* with a 72-h EC_{50} value of >21.9 mg/L and a 72-h NOEC of 10.3 mg/L reported. ECOSAR values for isobutyl isobutyrate were calculated to be 9.455 mg/L for fish, 27.556 mg/L for daphnids, and 0.771 mg/L for green algae. ECOSAR values for 2-ethylhexyl acetate were calculated to be 3.057 mg/L for fish, 3.571 mg/L for daphnids, and 0.260 mg/L for green algae. Data for 2-ethylhexyl acetate can be used to estimate the acute toxicity of isobutyl isobutyrate to fish and algae.

Exposure

Isobutyl isobutyrate occurs naturally in fruits and essential oils. Environmental concentrations of isobutyl isobutyrate may also occur from waste streams during manufacture or through solvent use in lacquers and thinners. Workplace exposure may occur via inhalation or dermal contact. Exposure during manufacture is limited by the enclosed nature of the process and by bulk handling and good manufacturing practices. Industrial and occasional consumer exposure can occur both dermally and via inhalation during application of lacquer and thinner formulations containing isobutyl isobutyrate. General population exposure can occur through inhalation of ambient air that may contain low concentrations resulting from industrial or commercial releases. General population exposure also occur through ingestion of foods containing isobutyl isobutyrate either naturally or as a synthetic flavorant (21 CFR § 121.1164).

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemical is currently of low priority for further work due to its low hazard profile.

Environment: The chemical possesses properties indicating a hazard for the environment (fish and algae). However, the chemical is of low priority for further work due to ready biodegradability and limited potential for bioaccumulation.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number:	97-85-8
IUPAC Name:	Isobutyl isobutyrate
Molecular Formula:	C8 H16 O2
Structural Formula:	$(CH_3)_2CHCOOCH_2CH(CH_3)_2$
Molecular Weight:	144.21g/mol
Synonyms:	Isobutyl 2-methylpropanoate 2-Methylpropyl 2-methyl propanoate IBIB

1.2 Purity/Impurities/Additives

No data available.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value
Physical state	Liquid at ambient temperature
Melting point	-80.7°C
Boiling point	148.6°C
Relative density	0.855 at 20°C
Vapour pressure	5.76 hPa at 25°C
Water solubility	520 mg/L at 27°C
Partition coefficient n-octanol/water (log value)	2.68 at 25°C calculated
Henry's law constant	1.58×10^{-3} atm-m^3/mol at 25°C

The references and reliability scores for the values found in Table 1 are in the Dossier.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Manufacture

Isobutyl isobutyrate is produced by a single manufacturer in the United States by the catalyzed esterification of isobutyric acid with isobutyl alcohol, using a continuous enclosed process. It is also produced as a co-product in the manufacture of Texanol® Ester Alcohol. In both cases the product is purified by distillation and stored in tanks. It is typically shipped via tank cars and tank

trucks (Eastman Chemical Company unpublished information (2003)). Current U.S. annual estimated production volume is about 4.1 thousand metric tons (< 9.0 million pounds). The non-confidential U.S. production range reported for the EPA TSCA Inventory Update Rule is 3.6-5.9 thousand metric tons (8-13 million pounds) in 2002. It is sold primarily in the United States except for about 300 metric tons, which are exported to Western Europe, Japan and Latin America. The sponsors are not aware of any isobutyl isobutyrate manufacture outside of the United States.

Use

Isobutyl isobutyrate is used principally as a solvent, especially for nitrocellulose lacquers and thinners as well as coatings for plastic substrates, and high-solids coatings. The majority of end uses are in coatings (automotive, industrial, wood furniture, and graphic arts). The concentration of isobutyl isobutyrate in these formulations typically ranges from 5-15% (Eastman Chemical Company unpublished information (2003)). It is also permitted by the Federal Food, Drug and Cosmetic Act for use as a flavorant in food (21 CFR 172.515 (4/1/90)), and is used as an insect repellent. (Sax, N.I. and R.J. Lewis, Hawley's Condensed Chemical Dictionary (1987)). It has a reported use in formulations of perfumes (Kirk-Othmer Encyclopedia of Chemical Technology (1990)).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Isobutyl isobutyrate occurs naturally in fruits and essential oils. Environmental concentrations of isobutyl isobutyrate may also occur from waste streams during manufacture. During solvent use in lacquers and thinners isobutyl isobutyrate will be released to air through evaporation.

2.2.2 Photodegradation

The photochemical removal of isobutyl isobutyrate from the troposphere occurs by reaction with hydroxyl radicals. This reaction is the rate-limiting step governing the overall residence time of isobutyl isobutyrate in air. Other processes, such as photolysis, wet deposition (rain-out), and dry deposition (aerosol formation) are not expected to play an important role in the atmospheric removal of isobutyl isobutyrate. Using a global average tropospheric hydroxyl radical concentration of 1.5×10^6 molecules/cm^3, a second order photo-oxidation rate constant of 5.4945×10^{-12} cm^3/molecule-sec, and assuming a 12-h daylight period, the half-life in air of isobutyl isobutyrate is expected to be about 1.947 days (Atkinson et al., 1987, EPISUITE v.3.11).

2.2.3 Stability in Water

The stability of isobutyl isobutyrate in water is pH dependent. At neutral pHs (pH 7) the half-life is about 9.217 years at 25°C. At pH 8, the hydrolysis half-life decreases to 337 days. Results were estimated using HYDROWIN v.1.67 (EPISUITE v.3.11).

2.2.4 Transport between Environmental Compartments

The values for the properties melting point, boiling point, vapor pressure, and aqueous solubility for isobutyl isobutyrate were taken from standard references (e.g., CRC Handbook, 1991-1992, Merck Index, Budvari, 1996) that contain no experimental details. Results of the use of the properties for the estimation of chemical transport between environmental compartments should be used with caution. The vapor pressure of isobutyl isobutyrate is 5.8 hPa at 25°C and the water solubility is 520 mg/L at 27°C. A Henry's law constant was calculated to be 1.58×10^{-3} atm-m^3/mol, using a

molecular mass of 144.21 g/mol and the preferred vapor pressure and water solubility. For chemicals with a Henry's Law constant <1.0 x 10^{-3} atm-m^3/mole, volatilization from water is expected to be moderate. Isobutyl isobutyrate is on the borderline of being moderately volatile.

The potential for isobutyl isobutyrate to volatilize from model rivers and lakes was calculated by EPISUITE (v.3.11) using a water solubility of 520 mg/L, a vapor pressure of 5.8 hPa, and a Henry's law constant of 1.58 x 10^{-3} atm-m^3/mol and default model assumptions. Volatilization half-lives from a model river and lake were 1.67 hours and 4.955 days, respectively. Thus, volatilization from rapidly flowing surface waters is an important removal process for isobutyl isobutyrate, while volatilization is only moderate from more quiescent lakes and other surface waters.

The preferred calculated log K_{ow} value is 2.68. This octanol/water partition coefficient suggests that isobutyl isobutyrate would partition moderately from water to soil, sediment, or biota. Using EPISUITE (v.3.11) and PCKOCWIN (v.1.66), the soil or sediment K_{oc} for isobutyl isobutyrate was calculated to be 53.3 L/kg based on the structural features of the molecule. This soil/sediment partitioning values indicate that isobutyl isobutyrate moves moderately through soil to groundwater, with some sorption to soil expected.

Fugacity modeling (Level III) was conducted for isobutyl isobutyrate using EPISUITE (v.3.11). Input parameters included molecular weight 144.21 g/mol, melting point -80.7°C, boiling point 148.6°C, water solubility 520 mg/L, log Kow 2.68, and Henry's law constant 1.58x10^{-3} atm-m^3/mol. Equal releases to air, water and soil were assumed. Media-specific half-lives were selected or calculated by the model. The model selected 5.495x10^{-12} cm^3/molecule-sec as the second order rate constant for atmospheric photo-oxidation (Atkinson, 1987). Biodegradation half-lives in water, soil and sediment (360 h, 360 h, and 1440 h, respectively) were selected by the model based on the biodegradation submodels within EPISUITE (v.3.11). All other parameters used were the model default values. The results support the above conclusions regarding the movement of isobutyl isobutyrate in the environment with 12.6% distributing to air, 34.4% to water, 52.7% to soil and 0.233% to sediment.

2.2.5 Biodegradation

Biodegradation data are not available for isobutyl isobutyrate. Data from analogous compounds can be used to estimate the biodegradability of isobutyl isobutyrate. Biodegradation studies with isopropyl acetate (CAS# 108-21-4) and isobutyl acetate (CAS# 110-19-0) are available. Using a standard method from the American Public Health Association (APHA, 1971), Price et al. (1974) reported 76% biodegradation of isopropyl acetate and 81% biodegradation of isobutyl acetate after 20 days, indicating that these analog compounds are readily biodegradable. These data with the analogous compounds would indicate that isobutyl isobutyrate would also likely to be readily biodegradable.

2.2.6 Bioaccumulation

The bioaccumulation potential of isobutyl isobutyrate is low to moderate. The calculated log K_{ow} value for isobutyl isobutyrate is 2.68. This octanol:water partition coefficient suggests that isobutyl isobutyrate would only accumulate in biological tissue to a moderate degree, but would not biomagnify in food chains. An estimated bioconcentration factor of 23.1 L/kg was calculated using the log Kow value of 2.68, which further suggests a low to moderate bioaccumulation potential (BCFWIN v.2.15, EPISUITE v.3.11).

2.2.7 Other Information on Environmental Fate

2.3 Human Exposure

Human exposure may occur during manufacture, formulation into products or during the use of product formulations containing isobutyl isobutyrate. Exposure also occurs through ingestion of foods that contain isobutyl isobutyrate, either naturally or as an intended food additive.

2.3.1 Occupational Exposure

No workplace exposure limits for isobutyl isobutyrate have been established. Limited exposure opportunity exists during production, which is by a single manufacturer using a closed continuous production process. The reactor, distillation column and storage tank are closed and connected via direct piping. The product is shipped primarily via tank car or tank truck, although smaller quantities may be shipped in drums. Exposure or release could occur during transport through an infrequent spill or accident. Occupational exposure during formulation of isobutyl isobutyrate solvent into products, such as lacquers and thinners is also likely to be limited, because the reactors used to mix up formulations are also enclosed. Exposure is more likely, by inhalation or through dermal contact, when products, such as lacquers or thinners, which contain isobutyl isobutyrate are used. Thirty-five personal samples were gathered on personnel working in the isobutyl isobutyrate manufacturing area. All samples were determined to be below 1 ppm (Eastman Chemical Company, 1984).

2.3.2 Consumer Exposure

Consumer exposure may occur during the use of product formulations that contain isobutyl isobutyrate. Such products include lacquers and thinners (Sax and Lewis, Hawley's Condensed Chemical Dictionary, 1981; Kuney, Chemcyclopedia,1990). End use data shows a 55% use in automotive coatings, a 43% use in general industrial coatings, and a 2% use in graphic arts and wood furniture (Eastman Chemical Company, 2003). Isobutyl isobutyrate's use as a solvent can result in release into the atmosphere through evaporation (SRC). General population exposure can occur through inhalation of ambient air containing low concentrations of this substance. Consumers also ingest foods that contain isobutyl isobutyrate naturally or that is added as a flavorant. Isobutyl isobutyrate has been identified as a volatile constituent of strawberries, cantaloupes and muskmelons (Yamashita et al., 1976; Dirinck et al., 1984; Yabumoto et al., 1977). Its presence reported to be found in the essential oil of hops and in vitis vinifera (Fenaroli, 1971). Of the 22 constituents isolated from Eriocephalus punctulatus oil, 50% were esters. Isobutyl isobutyrate was one of the most prevalent at 6.5% concentration (Roard et al., 1997).

Isobutyl isobutyrate is a food additive permitted for direct addition to food for human consumption (Federal Food, Drug and Cosmetic Act and Furia and Bellance ,1971). Synthetic flavoring substances and adjuvants, including isobutyl isobutyrate may be used in food under the Federal Food, Drug, and Cosmetic Act (Furia, Handbook of Food Additives. 2nd ed.,1968; 21 CFR §121.1164).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Data from isobutanol toxicity studies have been included in the human health section. Data from isobutanol are useful when assessing the hazards associated with the systemic toxicity of isobutyl

isobutyrate exposure due to the rapid and complete metabolism of isobutyl isobutyrate to isobutanol and isobutyric acid *in vivo*. Isobutanol is then further metabolized to isobutyric acid. Therefore, exposure to isobutyl isobutyrate via dermal, inhalation, and water or dietary administration is expected to result in the rapid appearance of isobutanol and isobutyric acid in the systemic circulation. Since exposure to either isobutyl isobutyrate or isobutanol results in systemic exposure to isobutanol and isobutyric acid, systemic toxicity data from studies that administer isobutanol directly are useful in identifying hazards associated with isobutyl isobutyrate exposure. Data from studies conducted with isobutyric acid were not included, since there were none available. The toxicokinetics of the metabolic reaction is documented and explained below.

3.1.1 Toxicokinetics, Metabolism and Distribution

Isobutyl isobutyrate metabolism has been studied in rats.

Studies in Animals

In vivo Studies

Metabolism/toxicokinetic studies have been conducted in male rats with isobutyl isobutyrate using intravenous injections (femoral vein) with simultaneous intravenous (jugular vein) sampling (Deisinger, unpublished, 2003). Isobutyl isobutyrate levels within the first 15 seconds had a mean value of 1045 µM and rapidly decreased thereafter and being undetected at 166 seconds. The calculated T1/2 by one-compartment modelling was 11.1 seconds. Isobutanol and isobutyric acid levels increased rapidly up to peak levels of 218 and 304 µM, respectively at 31-45 seconds. Isobutyric acid levels were consistently higher than isobutanol levels, suggesting further metabolism of the isobutanol metabolite to isobutyric acid. Both isobutanol and isobutyric acid levels remained increased throughout the 240 second sampling period.

Conclusion

Isobutyl isobutyrate is rapidly metabolised to isobutanol and isobutyric acid in rats.

3.1.2 Acute Toxicity

The acute toxicity values from the robust studies for all three routes of administration (oral, dermal, inhalation) are those conducted in 1956 at the Laboratory of Industrial Medicine of the Eastman Kodak Company. Although these studies were conducted prior to the promulgation of test guidelines, the design and conduct of these studies were judged acceptable by current standards.

Studies in Animals

Inhalation

Inhalation exposure to 5423 ppm (31.94 mg/L) of vapors of isobutyl isobutyrate for six hours killed 2 of 3 rats while 658 ppm (3.88 mg/L) killed 0 of 3 rats (Eastman Kodak Co., 1956). Prostration and narcosis was noted during exposure to the 5423 ppm. All of the animals that survived the 6-hour exposures gained weight at the end of the 14-day observation period. The toxicity data and clinical signs are in agreement with data from more recent isobutanol acute inhalation studies.

Dermal

The dermal LD50 values for isobutyl isobutyrate in guinea pigs was >10 ml/kg bw (Eastman Kodak Co., 1956).

Oral

The acute oral LD_{50} value in rats and mice was >6,400 mg/kg bw (Eastman Kodak Co., 1956). Weakness, ataxia, and death were noted at 12,800 mg/kg bw. The data from more recent isobutanol acute oral toxicity studies in the rat (conducted according to OECD TG 401 and GLP) are in broad agreement with the isobutyl isobutyrate data, giving values of around 3,000 mg/kg bw (Christopher, 1993).

Conclusion

The data from the acute toxicity studies with isobutyl isobutyrate suggest that it is of low acute toxicity to experimental animals via the oral, dermal, and inhalation routes of exposure.

3.1.3 Irritation

Skin Irritation

Studies in Animals

Isobutyl isobutyrate was reported to cause slight skin irritation (some redness and scaling of the skin) in guinea pigs when held in occlusive contact for 24 hours (Eastman Kodak Co., 1956). Current guidelines recommend a 4-hour contact time using a semi-occlusive covering.

Eye Irritation

No data available

Respiratory Tract Irritation

No data available

Conclusion

Isobutyl isobutyrate is a slight skin irritant when tested under conditions that were more stringent than current guidelines would recommend. There is no data for eye irritation.

3.1.4 Sensitisation

No data available

3.1.5 Repeated Dose Toxicity

Data from isobutanol toxicity studies have been included in this section. Data from isobutanol studies are useful for corroborating the isobutyl isobutyrate data due to the rapid and complete metabolism of isobutyl isobutyrate to isobutanol *in vivo*. Therefore, exposure to isobutyl isobutyrate via dermal, inhalation, and water or dietary administration results in the rapid appearance of isobutanol and isobutyric acid in the systemic circulation. Since exposure to either isobutyl isobutyrate or isobutanol results in systemic exposure to isobutanol and isobutyric acid, systemic toxicity data from studies that administer isobutanol directly are useful in corroborating studies conducted with isobutyl isobutyrate. The toxicokinetics of the metabolic reaction is documented and explained above. There are no repeated-dose toxicity studies conducted with isobutyric acid.

Studies in Animals

Oral

An 18-week subchronic oral gavage toxicity study with isobutyl isobutyrate, has been done in rats (15/sex/dose level) using dose levels of 0, 10, 100, or 1,000 mg/kg bw/day. Examination of blood and urine and the weights and microscopic structure of a limited range of organs were conducted (Drake, et al., 1978). The only change in haematological parameters observed was a decrease in hemoglobin in males at 100 and 1000 mg/kg bw/day after two weeks of exposure that were not observed at 6 and 18 weeks. At 1000 mg/kg bw/day, there was an increase in relative spleen weight that coincided with a decrease in relative body weights in males which was not considered significant. The NOAEL was 1000 mg/kg bw/day in rats. Oral gavage studies with isobutanol (13-week; 0, 100, 316, and 1000 mg/kg bw/day) in rats (30/sex/dose) broadly support the findings for isobutyl isobutyrate (TRL, 1987). Acute signs of toxicity (ataxia, hypoactivity) were noted immediately after oral exposure to 1,000 mg/kg day with isobutanol, but had largely resolved by the 4th week. No treatment-related effects were detected on organ weights or microscopic examinations of a range of tissues, and there were no clinical signs at the two lower doses. No clinical signs of exposure were noted following oral exposure to isobutyl isobutyrate. It was suggested that the difference in acute responses is related to the rate of absorption due to the vehicles used in each study. The isobutanol study used distilled water while the isobutyl isobutyrate study used a corn oil vehicle.

Conclusion

The NOAEL for oral exposure to isobutyl isobutyrate, in rats, is 1000 mg/kg bw/day.

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

Isobutyl isobutyrate was not mutagenic in a well-conducted standard plate incorporation Ames assay using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, or in Escherichia coli strain WP2 uvrA gene mutation assay (SafePharm, 2003). No *in vitro* mammalian cell assays were available.

In vivo Studies

There are no *in vivo* genotoxicity studies conducted with isobutyl isobutyrate. Data from a well-conducted isobutanol toxicity study has been included in this section. Data from isobutanol is useful when assessing the hazards associated with the *in vivo* genetic toxicity of isobutyl isobutyrate exposure due to the rapid and complete hydrolysis of isobutyl isobutyrate to isobutanol and isobutyric acid *in vivo*. There are no *in vivo* genetic toxicity studies conducted with isobutyric acid.

An oral *in vivo* mouse micronucleus test conducted with isobutanol is available from BASF Corporation (Engelhardt and Hoffman, 2000). In this well conducted study, isobutanol was administered once orally to male and female NMRI mice at doses up to 2,000 mg/kg/day body weight. The lowest level for producing toxicity was 1000 mg/kg bw/day with no effect on P/N ratio. Positive and negative controls all produced appropriate responses. Isobutanol did not produce any chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis (spindle poison effect).

Conclusion

Isobutyl isobutyrate was not genotoxic in *in vitro* experiments using bacterial cells and isobutanol was not genotoxic in *in vivo* experiments in mice.

3.1.7 Carcinogenicity

No adequate carcinogenicity studies are available for isobutyl isobutyrate or its metabolites.

3.1.8 Toxicity for Reproduction

Studies in Animals

Data from isobutanol reproductive and developmental toxicity studies have been included in this section. Data from isobutanol are useful when assessing the hazards associated with the reproductive and developmental toxicity of isobutyl isobutyrate exposure due to the rapid and complete hydrolysis of isobutyl isobutyrate to isobutanol and isobutyric acid *in vivo*. There are no reproductive or developmental toxicity studies conducted with isobutyric acid.

Effects on Fertility

A two-generation reproductive toxicity study has been conducted (according to US EPA Health Effects Test Guidelines OPPTS 870.3800) by inhalation with isobutanol (WIL Research Laboratory, 2003). Groups of male and female rats (30/sex/exposure concentration) were exposed by inhalation (6 hours/day, seven days/week) to 0, 1.52, 3.03, or 7.58 mg/L (500, 1000, or 2500-ppm) isobutanol for two generations from 10 weeks prior to mating. Daily treatments were continuous with the exception of the period between gestation day 21 through postnatal day 4 (removal of the dams from the pups during this period typically causes pup mortality). Exposure to 2500 ppm or 7.58 mg/L isobutanol (the NOAEL) did not cause any parental systemic, reproductive, or neonatal toxicity when administered for two generations via whole-body exposure.

Developmental Toxicity

In two definitive developmental toxicity studies (BASF, 1990 and Klimisch, 1990) conducted according to OECD TG 414, groups of pregnant female rats (25/group) or rabbits (15/group) were exposed via inhalation to 0, 0.5, 2.5 or 10 mg/L isobutanol for 6 hours/day during gestation (rats - days 6-15; rabbits – days 7-19). Rabbit dams exposed to 10 mg/L had slight decreases (not statistically significant) in body weight gain during gestation while exposures in rats had no treatment-related effects. No evidence of developmental or fetotoxicity was reported in either the rats or the rabbits fetuses. The NOAEL for isobutanol developmental toxicity was 10 mg/L.

Conclusion

Isobutyl isobutyrate is not expected to cause reproductive or developmental toxicity based upon the available data for isobutanol.

3.2 Initial Assessment for Human Health

Isobutyl isobutyrate exhibited low acute toxicity to experimental animals following acute oral, dermal, or inhalation exposure. Isobutyl isobutyrate is a slight skin irritant. Isobutyl isobutyrate is rapidly hydrolysed *in vivo* to isobutanol and isobutyric acid. Data from isobutanol acute oral toxicity and repeated-dose toxicity studies have been used to corroborate conclusions from studies conducted with isobutyl isobutyrate. In addition, *in vivo* mutagenicity, and reproductive and developmental toxicity studies have been used to identify hazards associated with isobutyl

isobutyrate exposure. Repeated dose toxicity studies for isobutyl isobutyrate using the oral route of exposure had NOAELs of 1000 mg/kg bw/day. The NOAEL for reproductive and developmental toxicity from a two-generation reproductive toxicity study and developmental toxicity studies (according to U.S. EPA and OECD test guidelines) with isobutanol in rats was approximately 2500 ppm 7.58 mg/L for the two-generation study and 10 mg/L for the developmental toxicity studies. A well conducted bacterial *in vitro* mutagenicity test with isobutyl isobutyrate was negative and an *in vivo* micronucleus test with isobutanol was negative for genotoxicity.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Analog Justification

The only acute toxicity data available for isobutyl isobutyrate is with the daphnid, *Daphnia magna*. Based on general structural similarities as acetates, data for 2-ethylhexyl acetate (CAS# 103-09-3) are used to address the aquatic toxicity for isobutyl isobutyrate.

Acute Toxicity Test Results

Fish

No data on fish toxicity are available for isobutyl isobutyrate. Data are available for the analogous compound 2-ethylhexyl acetate. Available data as well as ECOSAR predicted toxicity values are shown in Table 2 below. Two studies (conducted according to OECD guidelines) with rainbow trout (*Oncorhynchus mykiss*) are available for 2-ethylhexyl acetate (Hahne, 2002a; BASF AG, unpublished). Acute 96-hour LC_{50}s of 8.27 and >4.2 mg/L were reported in static renewal and flow through studies, respectively.

ECOSAR (v.0.99g, 2000) values for isobutyl isobutyrate and 2-ethylhexyl acetate are included for comparison to the available measured data. For isobutyl isobutyrate, an ECOSAR value of 9.455 mg/L for fish was calculated. An ECOSAR value of 3.057 mg/L was calculated for 2-ethylhexyl acetate.

Invertebrates

Data are available isobutyl isobutyrate and the invertebrate *Daphnia magna* from a guideline study, conducted according to GLP with reported 48-hour EC_{50} values of 55.8 to 59.3 mg/L reported (Patterson and Hirsch, 1995). For comparison purposes, a 48-h EC50 with Daphnia magna of 22.9 mg/L 2-ethylhexyl acetate is also presented (Hahne, 2002b). ECOSAR (v0.99g, 2000) values for isobutyl isobutyrate and 2-ethylhexyl acetate were 27.566 and 3.571 mg/L, respectively.

Green Algae

No acute toxicity data are available for green algae with isobutyl isobutyrate. Data are available from a guideline study conducted according to GLP with 2-ethylhexyl acetate and the green alga *Selenastrum capricornutum* with an acute 72-hour EC_{50} value of >21.9 mg/L reported and 72-h chronic NOEC (LOEC) values of 10.3 (21.9) mg/L (Hicks, 2002).

ECOSAR (v.0.99g, 2000) values for isobutyl isobutyrate and 2-ethylhexyl acetate were calculated to be 0.771 mg/L and 0.26 mg/L respectively. It should be noted that the ECOSAR value for algae for esters is generally not very predictive of measured values because ECOSAR is overly conservative in its predictions for green algae.

Aquatic Toxicity Summary

The data for isobutyl isobutyrate, the structural analog 2-ethylhexyl acetate and their ECOSAR values allow the estimation of the acute aquatic toxicity of isobutyl isobutyrate. Data for 2-ethylhexyl acetate can be used to predict the toxicity of isobutyl isobutyrate to fish and algae. The data from 2-ethylhexyl acetate suggests that isobutyl isobutyrate possesses properties indicating a hazard for fish and algae. All data are presented in Table 2.

Table 2 Aquatic toxicity data for isobutyl isobutyrate and its analogs.

	Isobutyl isobutyrate CAS# 97-85-8 C8H16O2 MW=144.22 g/mol	2-Ethylhexyl acetate CAS# 103-09-3 C10H20O2 MW=172.27 g/mol
Fish	No data available	8.27 mg/L >4.2 mg/L
Fish (ECOSAR)	9.455 mg/L	3.057 mg/L
Daphnids	55.8 to 59.3 mg/L	22.9 mg/L
Daphnid (ECOSAR)	27.566 mg/L	3.571
Green algae	No data available	>21.9 mg/L (72-h acute EC_{50}) NOEC (LOEC)= 10.3 (21.9) mg/L (72-h chronic)
Green algae (ECOSAR)	0.771 mg/L	0.260 mg/L

Chronic Toxicity Test Results

No data available.

4.2 Terrestrial Effects

No data available.

4.3 Other Environmental Effects

4.4 Initial Assessment for the Environment

The available physicochemical data are adequate to describe the properties of isobutyl isobutyrate. Isobutyl isobutyrate has a melting point of -80.7 ^{o}C, boiling point of 148.6 ^{o}C and has a vapor pressure of 5.8 hPa at 25^{o}C, a water solubility of 520 mg/L at 27^{0}C and a calculated log K_{ow} of 2.68. The photochemical removal of isobutyl isobutyrate as mediated by hydroxyl radicals occurs with a calculated half-life of 1.947 days. Isobutyl isobutyrate is likely to be readily biodegradable under aerobic conditions, based on data for isopropyl- and isobutyl-acetate. Isobutyl isobutyrate volatilises easily from moving rivers, but volatilises only moderately from quiescent lakes and other surface water bodies (calculated volatilization half-lives of 1.67 hours from a river and 4.955days from a lake). Isobutyl isobutyrate is not persistent in the environment and is not likely to bioaccumulate in

food webs. Based on Level III distribution modelling it is estimated that the majority of isobutyl isobutyrate released to the environment (equally to air, water, and soil) will partition into water (34.4%) and soil (52.7%), with a smaller amount in air (12.6%). The stability of isobutyl isobutyrate in water is pH dependent, at neutral pHs (7) the $T_{1/2}$ = 9.2 years at 25^0C and at higher pHs (8) the $T_{1/2}$ is shortened to 337 days.

Except for a study with the aquatic invertebrate *Daphnia magna*, aquatic toxicity data are not available for isobutyl isobutyrate. Data for the structurally similar 2-ethylhexyl acetate (CAS# 103-09-3) were used to supplement the data for isobutyl isobutyrate. For fish, two studies with rainbow trout (*Oncorhynchus mykiss*) and 2-ethylhexyl acetate are available. Acute 96-h LC_{50s} of 8.27 and >4.2 mg/L were reported for 2-ethylhexyl acetate. Data are available with isobutyl isobutyrate and the invertebrate *Daphnia magna* with 48-hour EC_{50} values of 55.8 to 59.3 mg/L reported. In addition, a daphnid study with 2 ethylhexyl acetate reported a 48-h EC_{50} of 22.9 mg/L. Data are available with 2-ethylhexyl acetate and the green alga *Selenastrum capricornutum* with a 72-h EC_{50} value of >21.9 mg/L and a 72-h NOEC of 10.3 mg/L reported. ECOSAR values for 2-ethylhexyl acetate were calculated to be 3.057 mg/L for fish, 3.571 mg/L for daphnids, and 0.260 mg/L for green algae. The results from 2-ethylhexyl acetate for acute toxicity to fish and algae can be used for the assessment of isobutyl isobutyrate. Terrestrial data are not available.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work for human health due to its low hazard profile. The chemical possesses properties indicating a hazard for the environment (fish and algae). However, the chemical is of low priority for further work due to ready biodegradability and limited potential for bioaccumulation.

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SIDS DOSSIER

for:

ISOBUTYL ISOBUTYRATE

.....

CAS No. 97-85-8

Sponsor Country: U.S.A.

DATE: August 2005

1.01 SUBSTANCE INFORMATION

A. CAS-Number

97-85-8

B. Name (IUPAC name)

Isobutyl Isobutyrate

C. Name (OECD name)

Isobutyl Isobutyrate

D. CAS Descriptor

Not applicable in this case

E. EINECS-Number

F. Molecular Formula

C8 H16 O2

G. Structural Formula

(CH3)2CHCOOCH2CH(CH3)2

H. Substance Group

I. Substance Remark

J. Molecular Weight

144.21 g/mol

1.02 OECD INFORMATION

A. Sponsor Country:

United States of America

B. Lead Organisation:

Name of Lead Organisation:	American Chemistry Council
Contact person:	Barbara Francis
Address:	1300 Wilson Blvd.

Arlington, VA 22209
U.S.A.
Tel: 703-741-5609
Fax: 703-741-6091

1.1 GENERAL SUBSTANCE INFORMATION

A. TYPE OF SUBSTANCE

Natural organic compound

B. **Physical State** (at 20°C and 1.013 hPa)
Liquid

C. **Purity** (indicate the percentage by weight/weight)
98.5445% (w/w) average purity
Reference: Eastman Chemical Company Product Quality Report (22 March 2005)

1.2 SYNONYMS

Isobutyl 2-methylpropanoate
2-Methylpropyl 2-methyl propanoate
IBIB

1.3 IMPURITIES

Isobutyl alcohol = 0.2% maximum
Water = 0.05% (w/w) maximum
Other minor impurities = ca 1% total
Reference: Eastman Chemical Company Product Quality Report (22 March 2005)

1.4 ADDITIVES

None

1.5 QUANTITY

3.6 – 5.9 thousand metric tons in 2002 in the U.S. only

8M – 13 Mlbs

1.6 LABELLING AND CLASSIFICATION

No data available

1.7 USE PATTERN

A. General

Type of Use: **Category: Closed process**

Industrial, commercial solvent, automotive coatings, industrial coatings, graphic arts, wood furniture

Type of Use: **Category: Wide dispersive**

B. Uses in Consumer Products

Food flavorant, insect repellent, found in perfumes

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

Remark: Thirty-five personal samples were gathered on personnel working in the isobutyl isobutyrate manufacturing area. All samples were determined to be below 1 ppm.

Reference: Eastman Chemical Company, Industrial Hygiene Department. Unpublished communications. February-March, 1984.

1.9 SOURCES OF EXPOSURE

From industrial waste streams, evaporation during use as a solvent, natural occurrence in fruits and essential oils.

1.10 ADDITIONAL REMARKS

No additional remarks

2.1 MELTING POINT

(a) Preferred value

Value:	-80.7 ^{0}C
Method:	other: not reported
GLP:	no data
Test substance:	Isobutyl Isobutyrate [CAS #97-85-8]; colorless liquid (Hawley's Condensed Chemical Dictionary. 1987.) 25.10.2000
Reliability:	Score=2 valid with restrictions, lack of method details
Flag:	Critical study for SIDS endpoint
Reference:	CRC Handbook of Chemistry and Physics. 1991-1992. D.R. Lide (ed.) 72nd ed. CRC Press, Inc. Boca Raton, FL. P.3-155. Hawley's Condensed Chemical Dictionary. 1987. N.I. Sax and R.J. Lewis, Sr. (eds.). 11th ed. Van Nostrand Reinhold Co. New York, NY. P.654.

2.2 BOILING POINT

(a) Preferred value

Value:	148.6 ^{0}C
Method:	other: not reported
GLP:	no data
Test Substance:	Isobutyl isobutyrate [CAS#97-85-8]; colorless liquid (Hawley's Condensed Chemical Dictionary. 1987.) 25.10.2000
Reliability:	Score=2 valid with restrictions, lack of method details
Reference:	CRC Handbook of Chemistry and Physics. 1991-1992. D.R. Lide (ed.) 72nd ed. CRC Press, Inc. Boca Raton, FL. P.3-155.

(b)

Value:	147-148 ^{0}C at 1013.25 hPa
Method:	other: not reported
GLP:	no data
Test Substance:	Isobutyl isobutyrate [CAS#97-85-8]; colorless liquid (Hawley's Condensed Chemical Dictionary. 1987.) 25.10.2000
Remark:	Reported as 147-148 deg. C @ 760 mm Hg
Reliability:	Score = 2, valid with restrictions, lack of method details
Flag:	Critical study for SIDS endpoint
Reference:	Sigma-Aldrich Product Information. http://www.sigma-aldrich.com

2.3 DENSITY

(a) Preferred result

Type:	density
Value:	0.855 g/cm^3 at 20^{0}C
Method:	other: not reported
Test Substance:	Isobutyl isobutyrate [CAS#97-85-8]; colorless liquid, .97% pure (confirmed by GC)
Reliability:	Score=2 valid with restrictions, lack of method details
Reference:	Merck-Schuchardt. Hohenbrunn, Germany. Safety Data Sheet. Isobutyl isobutyrate for synthesis. 04.2000. the Merck ChemicalDatabase-online. http://www.merck.de

(b) Type: Relative density

	Value:	0.855
	Method:	other: not reported
	GLP:	no data
	Test substance:	Isobutyl isobutyrate [CAS #97-85-8]; liquid, 99% pure
	Reliability:	Score = 2, valid with restrictions, lack of method details
	Reference:	Sigma-Aldrich Product Information http://www.sigma-aldrich.com
(c)	Type:	relative density
	Value:	0.875 at 4^0C
	Method:	other: not reported
	GLP:	no data
	Test substance:	Isobutyl isobutyrate [CAS #97-85-8]; colorless liquid (Hawley's Condensed Chemical Dictionary. 1987.)
	Remark:	Value also reported for test conducted @ 0 deg. C
	Reliability:	Score = 2, valid with restrictions, lack of method details
	Reference:	CRC Handbook of Chemistry and Physics. 1991-1992. D.R. Lide (ed.) 72nd ed. CRC Press, Inc. Boca Raton, FL. P.3-155.

2.4 VAPOUR PRESSURE

(a)	Preferred value	
	Value:	5.76 hPa at 25^0C
	Method:	other (measured): not reported
	GLP:	no data
	Remark:	Reported as 4.33 mm Hg at 25° C
	Test substance:	Isobutyl Isobutyrate [CAS# 97-85-8]
	Reliability:	Score=2 valid with restrictions, lack of method details
	Flag:	Critical study for SIDS endpoint
	Reference:	Daubert, T.E. and R.P. Danner. 1989. Physical and Thermodynamic Properties of Pure Chemicals. Data Compilation. Hemisphere Publishing Corp. New York, NY.

2.5 PARTITION COEFFICIENT LOG10POW

(a)	Preferred value	
	Log pow:	2.68 at 25^0C
	Method:	other (calculated): Atom/fragment contribution method; KOWWIN
	GLP:	no
	Test substance:	Isobutyl isobutyrate [CAS # 97-85-8]
	Reliability:	Score=2 valid with restrictions, calculated value
	Flag:	Critical study for SIDS endpoint
	Reference:	KOWWIN, v1.67. Log Octanol-Water Partition Coefficient. EPISUITE Version 3.10. Syracuse Research Corporation. Syracuse, NY.

2.6 WATER SOLUBILITY

(a)	Preferred value	
	Value:	520 mg/L at 27±0.5° C
	Qualitative:	Moderately soluble (100-1000 mg/L)
	Method:	The slow-stir method described by Ellington (1999) was used. Duplicate glass tanks (20-L) were filled with 20 L deionised water, covered with glass, and sealed with parafilm. Test substance was placed in the tank by slow introduction via pipette. The tank was placed on a stir plate and stirred

continuously with a Teflon stir bar at a rate that no vortex was observed. Sample aliquots were collected on days 0, 3, 4, 7, 8, 9, 10, and 14, extracted in methanol and analysed using GC-FID.

GLP: yes

Results: pH values of samples were 5.2±0.7. Daily results are shown in the table as follows:

Day	Slow-stirring Tanks	
	(Mean concentration, mg/L)	
Blank tanks	<2.0	<2.0
3	*423.4*	*455.2*
4	*485.4*	518.8
7	546.7	525.8
8	526.9	511.0
9	506.2	533.8
10	495.7	496.6
14	517.5	537.4
Mean	520.0	

Data in italics font not used to calculate the mean water solubility.

Test substance: other TS, Isobutyl isobutyrate [CAS #97-85-8]
Reliability: (1) valid without restrictions
Flag: Critical study for SIDS endpoint
Reference: Ellington JJ. (2003) J Chem Eng Data. 44: 1414-1418
Hoffman CM (2003) Isobutyl Isobutyrate – Water Solubility. Final Report # 1727-WS, Eastman Kodak Company, Rochester, NY.

(b) Value: 1000 mg/L at 20^0C
Qualitative: moderately soluble (100-1000 mg/L)
Pka: at 25 ^{0}C
Method: other: stated to be measured, details not given
GLP: no data
Test substance: Isobutyl isobutyrate [CAS #97-85-8]
Reliability: Score=2 valid with restrictions, lack of method details
Reference: Flick, E.W. 1991. Industrial Solvents Handbook. 4th ed. Noyes Data Corp. Park Ridge, NJ. P.815.

(c) Value: 5000 mg/L
Qualitative: soluble (1000-10000 mg/L)
Pka:
PH:
Method: other: not reported
GLP: no data
Test substance: Isobutyl isobutyrate
Remark: Reported as 0.5 g/100 mL
Reliability: Score = 2, valid with restrictions, lack of method details
Reference: Industrial Hygiene and Toxicology. 1989. F. Patty (ed.). 2nd ed. Volume II: Toxicology. Interscience Publishers. New York, NY. P. 1866.

(d) Value: 670.5 mg/L at 25^0C
Qualitative: moderately soluble (100-1000 mg/L)
Pka:
PH:
Method: other, calculated, WSKOW

	GLP:	no
	Test substance:	Isobutyl isobutyrate
	Remark:	Log Kow used for calculation = 2.68 (estimated); melting point used for calculation = -80.7 deg. C
	Reliability:	Score=2, valid with restrictions, calculation procedure
	Reference:	WSKOW, v1.41. Water Solubility Estimate from Log Kow. EPISUITE Version 3.11. Syracuse Research Corporation. Syracuse, NY.
(e)	Value:	1273 mg/L at 25°C
	Qualitative:	moderately soluble (100-1000 mg/L)
	Pka:	
	PH:	
	Method:	Other, calculated, WATERNT
	GLP:	No
	Test Substance:	other, TS, Isobutyl Isobutyrate
	Remark:	Structural fragment method
	Reliability:	Score = 2, valid with restrictions, calculation procedure
	Reference:	WATERNT, 1.01, EPI Suite v.3.11, United States Environmental Protection Agency, 2003.

2.7 FLASH POINT *(LIQUIDS)*

(a)	Preferred value	
	Value:	37.2°C
	Method:	other: not reported
	Test substance:	Isobutyl isobutyrate; liquid, 99% pure
	Reliability:	Score=2, valid with restrictions, lack of method details
	Reference:	Sigma-Aldrich Product Information http://ww.sigma-aldrich.com
(b)	Value:	38°C
	Method:	other: not reported
	GLP:	no data
	Test substance:	other TS Isobutyl isobutyrate; colorless liquid, .97% pure (confirmed by GC)
	Reliability:	Score = 2, valid with restrictions, lack of method details
	Reference:	Merck-Schuchardt. Hohenbrunn, Germany. Safety Data Sheet. Isobutyl isobutyrate for synthesis. 04.2000. The Merck Chemical Database-online. http://www.merck.de

2.8 AUTO FLAMMABILITY *(SOLID/GASES)*

No data available

2.9 FLAMMABILITY

No data available

2.10 EXPLOSIVE PROPERTIES

No data available

2.11 OXIDIZING PROPERTIES

No data available

2.12 ADDITIONAL REMARKS

No additional remarks

2.13 ADDITIONAL DATA

No additional data

3.1 STABILITY

3.1.1 PHOTODEGRADATION

(a)	Type:	other: see remarks
	Light source:	
	Light spect.:	nm
	Rel. intensity:	based on Intensity of Sunlight
	Deg. Product:	N/A
	Method:	EPIWIN Program
	Year:	N/A
	GLP:	No
	Test substance:	Isobutyl isobutyrate
	Remark:	If released to the atmosphere, isobutyl isobutyrate is expected to degrade by reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant was calculated as 5.49E-12 cm3/(molecules-sec) at 25°C. Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated half-life is 1.947 days.
	Reliability:	Score = 2, valid with restrictions, calculation procedure
	Flag:	Critical study for SIDS endpoint
	Reference:	Atkinson, R. 1987. A Structure-activity relationship for the estimation of rate constants for the gas-phase reaction of OH radicals with organic compounds. J. Inter. Chem. Kinet. 19:799-828. AOPWIN, v1.91. Atmospheric Oxidation. EPI Suite Version 3.11. U.S. Environmental Protection Agency, 2003.

3.1.2 STABILITY IN WATER

(a)	Preferred result	
	Type:	abiotic
	T1/2 pH4:	
	T1/2 pH7:	9.217 year at 25 degree C
	T1/2 pH9:	
	T1/2 pH8:	336.6 day at 25 degree C
	Deg. Product:	
	Method;	other (calculated): HYDROWIN
	Year:	
	GLP:	no
	Test substance:	other TS Isobutyl isobutyrate
	Remark:	Rate constant for pH.8 @ 25 deg. C = 2.383e-2 L/mol*sec.
	Reliability:	Score=2 valid with restrictions, calculation procedure
	Flag:	Critical study for SIDS endpoint
	Reference:	HYDROWIN, v1.67. Aqueous Base/Acid-Catalyzed Hydrolysis. EPI Suite, v. 3.11, U.S. Environmental Protection Agency, 2003.

3.1.3 STABILITY IN SOIL

No data available

3.2 MONITORING DATA (ENVIRONMENT)

No data available

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

(a)	Type:	Volatilization from surface waters
	Test substance:	Isobutyl isobutyrate
	Method:	Calculated using EPISUITE v3.11 (USEPA, 2003)
	Result:	Half-life from model river: 1.67 hours Half-life from model lake: 4.955 days
	Remark:	Based on Henry's law constant of 1.58E-3 atm-m3/mol, vapor pressure of 4.33 mm Hg, water solubility of 520 mg/L, and a molecular weight of 144.21 g/mole, and model defaults (for model river: river 1m deep, water flow at 1 m/sec, wind speed of 5 m/sec; for model lake: 1 m deep, water flow 0.05 m/sec., wind speed 0.5 m/sec).
	GLP:	not applicable
	Reliability:	Score = 2, valid with restrictions, calculation procedure
	Reference:	EPISUITE v3.11, U.S. Environmental Protection Agency (2003).
(b)	Type:	soil or sediment partition coefficient (Koc)
	Test substance:	Isobutyl isobutyrate
	Method:	Calculated using EPISUITE v.3.11 and PCKOCWIN v.1.66 using structural features of the molecule.
	Result:	53.3 L/kg
	GLP:	not applicable
	Reliability:	Score = 2, valid with restrictions, calculation procedure
	Reference:	PCKOWIN. Version 1.66. Soil Adsorption Coefficient. EPI Suite, v. 3.11, U.S. Environmental Protection Agency, 2003.
(c)	Type:	Henry's Law Constant
	Test substance:	Isobutyl isobutyrate
	Method:	Calculated using water solubility 520 mg/L, vapor pressure 4.33 mm Hg, and molecular weight 144.21 g/mol.
	Result:	1.58E-4 atm-m3/mol
	GLP:	not applicable
	Reliability:	Score = 2, valid with restrictions, calculation procedure
	Reference:	EPI Suite, v. 3.11, U.S. Environmental Protection Agency, 2003.

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

(a)	Preferred result	
	Type:	fugacity model level III
	Media:	other: air, water, soil, and sediment
	Air (level III):	12.6%
	Water (level III)	34.4%
	Soil (level III):	52.7%
	Sediment (level III)	0.233%
	Method:	other

Year:	
Remark:	Properties used were: melting point –80.7° C, boiling point 148.6°C, vapor pressure 4.33 mm Hg at 25°C, log Kow 2.68, water solubility 520 mg/L, Henry's Law Constant 1.58E-3 atm m3/mol. All other model parameters were default values.
Remark:	Air: half-life = 46.7 hr, emissions = 1000 kg/hr Water: half-life = 360 hr, emissions = 1000 kg/hr Soil: half-life = 360 hr, emissions = 1000 kg/hr Sediment: half-life = 1440 hr, emissions = 0 kg/hr Persistence Time: 194 hr
Reliability:	Score = 2, valid with restrictions, calculated
Flag:	Critical study for SIDS endpoint
Reference:	Level III Fugacity Model. EPI Suite, v. 3.11, U.S. Environmental Protection Agency, 2003.

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

3.5 BIODEGRADATION

No data were available for isobutyl isobutyrate, so data for isopropyl and isobutyl acetate, are presented as analog compounds. The biodegradability of isobutyl isobutyrate (C4) is expected to be similar to isobutyl acetate (C4).

(a) Preferred value

Type:	aerobic
Inoculum:	domestic sewage, non adapted
Concentration:	3, 7, and 10 mg/L (at least two of these were tested in duplicate)
Contact time:	20 days
Degradation:	76% after 20 days
Result:	readily biodegradable
Kinetic of test subst.	5 day = 61%, 10 day = 72%, 15 day = 74%, 20 days = 76%
Method:	BOD (Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edi. American Public health Association, New York, NY)
Method:	Settled domestic wastewater was filtered through glass wool and added (3 mL/bottle) to clean 300 mL BOD bottles. Aerated dilution water containing minerals specified in the method were added to the bottles along with buffer. Test chemical was added to the bottles. Potential oxygen demand was 3 to 30 mg/L over 20 days. Dissolved oxygen was measured on days 0, 5, 10, 20 using a dissolved O2 meter. When oxygen decreased to <4 mg/L in any bottle, it was reaerated.
Year:	1971
GLP:	no data
Test substance:	isopropyl acetate (CAS #108-21-4)
Remark:	Typical unacclimated biodegradation curves for acetates were provided. The biodegradation curve for isopropyl acetate showed steadily increasing oxidation from test initiation to Day 10, followed by a plateauing through day 20. isopropyl acetate is readily biodegradable. Measured COD was reported as 1.67 mg/mg; the theoretical oxygen demand was reported as 2.04 mg/mg.
Reliability:	Score = 2, valid with restrictions, not all procedures mentioned
Reference:	Price, K.S., G.T. Waggy, and R.A. Conway. 1974. Brine Shrimp Bioassay and Seawater BOD of Petrochemicals. J. Water Pollut. Contr. Fed. 46:63-77.

(b)	Type:	aerobic
	Inoculum:	domestic sewage, non-adapted
	Concentration:	3, 7, and 10 mg/L (at least two of these were tested in duplicate)
	Contact time:	20 days
	Degradation:	81% after 20 days
	Results:	5 day = 60%, 10 day = 74%, 15 day = 79%, 20 days = 81%
	Method:	BOD (Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edi. American Public Health Association, New York, NY)
	Year:	1971
	GLP:	no data
	Test substance:	Isobutyl acetate (reported as pure chemical)
	Remark:	Typical unacclimated biodegradation curves for acetate esters were provided. The biodegradation curve for isobutyl acetate showed steadily increasing oxidation from test initiation to Day 20. Greater than 50% was biodegraded in 20 days; therefore, isobutyl acetate is expected to biodegrade in the environment based on a standard BOD study. Measured COD was reported as 1.43 mg/mg; the theoretical oxygen demand was reported as 2.20 mg/mg.
	Reliability:	Score = 2, Valid with restrictions, not all procedures mentioned
	Flag:	Critical study for SIDS endpoint
	Reference:	American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edi, New York, NY. Price, K.S., G.T. Waggy, and R.A. Conway. 1974. Brine Shrimp Bioassay and Seawater BOD of Petrochemicals. J. Water Pollut. Contr. Fed. 46:63-77.

3.6 BOD_5,COD OR RATIO BOD_5/COD

No data available

3.7 BIOACCUMULATION

(a)	BCF:	12.2
	Elimination:	
	Method:	other: calculated
	Year:	
	GLP:	no
	Test substance:	isobutyl isobutyrate
	Remark:	The BCF was calculated using a water solubility of 1000 mg/L at 20°C (Flick, 1991) and a recommended linear regression-derived equation (Lyman, et al. 1990). Based on the estimated BCF, isobutyl isobutyrate is not expected to bioaccumulate in fish or other aquatic organisms (Syracuse Research Corporation).
	Reliability:	Score = 2, Valid with restrictions, calculation method
	Reference:	Lyman, W.J., W.F. Reehl, and D.H. Rosenblatt. 1990. Handbook of Chemical Property Estimation Methods. American Chemical Society. Washington, D.C.
(b)	BCF:	23.1
	Elimination:	
	Method:	other: calculated, BCFWIN
	Year:	
	GLP:	no
	Test substance:	Isobutyl isobutyrate

Remark:	Log Kow used in calculation = 2.68 (estimated)
Reliability:	Score = 2, Valid with restrictions, calculated procedure
Reference:	BCFWIN v.2.15, EPISUITE v. 3.11 (U.S. Environmental Protection Agency, 2003).

3.8 ADDITIONAL REMARKS

A. Sewage Treatment

No data available

B. Other

No data available

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Analog Justification
No ecotoxicity studies with fish or algae are available for isobutyl isobutyrate. Data for the structurally similar 2-ethylhexyl acetate (CAS# 103-09-3) are presented to support the endpoints for isobutyl isobutyrate.

(a)

Remark:	Due to the absence of acute fish toxicity data for isobutyl isobutyrate, data for a structural analog are used (2-ethylhexyl acetate, CAS# 103-09-3).
Preferred Value	
Type:	static renewal
Species:	*Oncorhynchus mykiss* (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/L
LC50:	8.27
Limit test:	no
Analyt. monitoring:	yes
Method:	OECD guideline 203 (specific protocol titled, "Acute Toxicity of the Water Accomodated Fraction (WAF) for 2-Ethylhexyl Acetate to the Rainbow Trout, Oncrhynchus mykiss, Determined Under Static Test Conditions, and Amendment"
Year:	2002
GLP:	yes
Test substance:	2-ethylhexyl acetate (CAS RN 103-09-3), analog for isobutyl acetate, 99.7% purity, source: Aldrich Chemical Co., Milwaukee, WI, USA.
Method:	Rainbow trout were obtained as eyed embryos from a commercial supplier. Fish were maintained in blended laboratory freshwater. After hatch and swim-up and absorption of the yolk sac, fish were fed brine shrimp and commercial fish food twice daily. A subset of fish were removed from the main population and acclimated to test dilutio water temperature (~15 deg. C) for 72 hours before testing Fish used in the toxicity tests were about 130 days old, had a mean length of 39 mm and a mean weight of 0.368 g. Fish were not fed during the test. The toxicity test was conducted using 4-L vessels. Test volume was 3 L. During testing, vessels were place in a 15±1 deg. C water bath. Light was maintained on a 16h daylight cycle. Fish were not fed during the test. No aeration was employed during the test. The control/dilution water was a combination of naturally hard well water and well water that was de-mineralized by reverse osmosis yielding the desired range of hardness (total hardness ranging from 130 to 160 mg/L as CaCO3). The test employed a water accomodated fraction approach (WAF) in which an 9.0 gram of test material was placed on the surface of water in a 9.5 liter carboy filled with 9 liter dilution water. The solution was stirred for 20 hours with a stir bar creating a vortex <25% solution depth. After stirring the solution was allowed to separate for one hour. The aqueous solution was siphoned from the bottom of the carboy for use. Fresh solutions were prepared daily. Test concentrations were analyzed daily by gas chromatography (GLC) equipped with a flame ionization detector (GC-FID).

		The test was conducted in 4 liter glass jars. Two replicates of 5 fish each were exposed to the control/dilution water and to each of six dilutions of the WAF solution (1.6, 3.3, 6.5, 13, 25, 50% WAF). Renewals were made daily. Mortality and abnormal signs of behavior were recorded daily.
	Results:	Water quality parameters measured during the test included: total hardness, 164 mg/l (as CaCO3); alkalinity, 162 mg/l (as CaCO3); dissolved oxygen ranged from ~100% saturation at renewal of test solution to 45% saturation mg/l; temperature ranged from 14 to 16 deg. C; and pH ranged from 7.61 to 8.26. Test solutions were observed to be clear and colorless with no visible particulates, surface film, undissolved test substance, or precipitate. Measured concentrations in the new solutions ranged from 0.41 to 16.7 mg/L across treatments. Measured concentrations in old solutions (24-h) ranged from <0.347 to 13.8 mg/L. Mean measured concentrations (using ½ the MDL for non-detects) were 0.284, 0.570, 1.34, 2.51, 6.42, and 15.3 mg/L across all treatments. Results of the 96-h acute toxicity test, by concentration level tested: Control: No mortality 0.284 mg/L: No mortality or sublethal effects 0.57 mg/L: No mortality or sublethal effects 1.34 mg/L: No mortality or sublethal effects 2.51 mg/L: No mortality or sublethal effects 6.42 mg/L: Partial mortality (20%) at 6.42 mg/L at 96 hours, all remaining fish (8/10) showed sublethal effects; 15.3 mg/L: 100% mortality in both replicates at 24 hours. The 96-h LC_{50} was reported as 8.27 mg/l using the Trimmed Spearman-Karber method. Confidence limits were 6.58 to 10.4 mg/L.
	Reliability:	Score = 1, valid without restrictions
	Reference:	Hahne R. 2002a. Acute Toxicity of the Water Accommodated Fraction (WAF) for 2-Ethylhexyl Acetate to the Rainbow Trout, *Oncorhynchus mykiss*, Determined under Static Test Conditions. Report #46955 prepared by ABC Laboratories Inc., Columbia Missouri. May 22, 2002.
(b)	Type:	flow through
	Species:	*Oncorhynchus mykiss* (Fish, fresh water)
	Exposure period:	96 hour(s)
	Unit:	mg/L
	Analytical monitoring:	yes
	NOEC:	> 4.2
	LC0:	> 4.2
	LC50:	> 4.2
	LC100:	> 4.2
	Limit Test:	yes
	Method:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
	Year:	1992
	GLP:	yes
	Test substance:	other TS: 2-ethylhexyl acetate (BASF), purity : 99.3 %

Result:	In conclusion the 96 hour LC_{50} values for 2-Ethylhexylacetate in the Rainbow trout (Oncorhynchus mykiss) were >4.5 mg/L based on the nominal concentration of the test substance and >4.2 mg/L based on the mean of analytically determined concentration. No lethality was observed up to the solubility limit of the test substance in water (3.9 mg/L).
Test conditions:	0, 4.5 mg/L based on technical test substance. The test was carried out slightly above the solubility limit of the test substance in water (3.9 mg/L).
Test organisms	
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Animal supplier:	Forellenzucht Trostadt GbR, Dorfstrasse 7, 98646 Trostadt, Germany
Body weight:	(wet weight): 2.08 g (1.21 - 3.46 g)
Body length	(from the top of the snout to the end of the caudal fin): 6.1 cm (5.1 - 7.0 cm)
Age of the animals:	approx. 5 months
Hatching date:	Sep 08, 2003
Arrival at the testing facility:	Dec 04, 2003
Remark:	All animals used for the study were derived from the same batch of fish. All test animals were observed for their health state during insertion into the test vessels. No signs for sickness, injuries or abnormalities were observed. EXPERIMENTAL PROCEDURE 1. Acclimatization Duration of acclimatization to testing conditions including light regime: 14 days, acclimatization to test water and temperature: 8 days Photoperiod: 16 hours light, 8 hours dark Water quality: Non chlorinated charcoal filtered tap water (Frankenthal, Germany) mixed with deionized water Total hardness:Approx. 1.0 mmol/L = 100 mg/L CaCO3 Acid capacity: Approx. 2.2 mmol/L Oxygen content:> 80% saturation pH-value: Generally 7.5 - 8.5 Temperature: Approx. 15°C (8 days) Diet: Ecostart 17 (Bio Mar), supplier Kofu Tiernahrung, Betriebsstätte Wesel, Hafenstr. 11 - 13, 46483 Wesel, Germany, ad libitum, additionally generally on workdays frozen brine shrimp (artemia). Withdrawal of feed: During the last day before start of exposure Medical treatment: None during acclimatization Mortality during the last week before start of exposure: 0 % Test water Water quality: Non chlorinated charcoal filtered tap water (Frankenthal, Germany, aerated) mixed with deionized water Total hardness: Approx. 1.0 mmol/L = 100 mg/L CaCO3 Conductivity: Approx. 250 µS/cm (at 25°C) Ca content: Approx. 40 mg/L Mg content: Approx. 5 mg/L Acid capacity: Approx. 2.2 mmol/L pH-value: Generally 7.7 - 8.1 Temperature: Approx. 15°C

The tap water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Technical Services of BASF Aktiengesellschaft as well as for presence of microbes by a contract laboratory. On the basis of the analytical findings, the drinking water was found to be suitable for toxicity tests. German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetzblatt, December 05, 1990) served as a guideline for maximum tolerable contaminants. The individual results are to be found in the archives of the testing facility.

Preparation of the test concentrations:
The test was conducted in a flow through system. The flow-through-system started 3 days before the fishes were placed into the test vessels to ensure that the test system was saturated with the test substance. The stock solution was prepared in the following way: The test substance was weighed into a volumetric flask. The volumetric flask was made up with acetone. The stock solution was completely dissolved and clear. The stock solution was transferred into a 50 mL glass injector which was attached to a perfusion pump. The stock solution was continuously injected into the mixing vessel, where it was admixed to a continuous flow of dilution water. The mixture of dilution water and the stock solution of the test substance was flowing continuously into the test vessels. The flow rate of the dilution water was calibrated (maximum deviation less than 10%) before the system was started. The test vessels were temperated by the dilution water. The apparatus was checked daily for proper function. The flow meter (rotameter) levels were checked daily and corrected if necessary. The control group was performed as solvent control with the same concentration of acetone as the concentration group. A stock solution of 62.5 mL acetone in 2 L dilution water was mixed at a flow rate of 60 mL / hour with 18.69 L dilution water / hour. The concentration of acetone in all test vessels was 0.1 mL acetone / L test solution.

The nominal test concentration was 4.5 mg/L.

Test conditions
The exposure was carried out in a flow-through system.

Test vessels: glass aquaria (59 cm long, 28.5 cm wide, 30 cm high) with an overflow 26.5 cm above the bottom, overflow covered with plastic gauze
Volume of water: 45 L
Flow rate: 18.75 L / h
Volume of exchange/day: Approx. 10
Animals / test vessel: 10
Loading (g fish/L water): 0.5
Loading (g fish/L water/day): 0.05
Test vessel / concentration: 1
Photoperiod: 16 hours light, 8 hours darkness
Light intensity: Approx. 82 - 280 Lux (the light intensity is determined in regular intervals at the surface of the aquaria)
Temperature: 15°C
Aeration: None
Feeding: None

Insertion of test organisms

The test organisms were introduced into the test vessels according to a randomization plan prepared by the testing laboratory using a program of the laboratory data evaluation group of the testing facility.

Observations
Symptoms and lethality:
The fish were observed for survival and toxic signs changes in appearance, swimming behavior and behavior in comparison to the control group) within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure. Fish were considered dead if there was no visible movement and no reaction after touching. Dead fish were removed from the test vessels.

Water parameter:
Temperature, oxygen content and pH-value were measured in each test vessel within 1 hour after start of exposure and after 24, 48, 72 and 96 hours.

Analytical determinations
An analytical method for the determination of the test substance in the test water was developed by the Analytical Department of BASF Aktiengesellschaft. The analyses were carried out at the Analytical Chemistry Laboratory of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft under the responsibility of Dr. E. Leibold / Lan Ma in compliance with the Principles of Good Laboratory Practice. For methods and details see Appendix.

Stability in water: See analytical concentration control
Samples for analytical determination of the concentration were taken within the first hour after the start of exposure, after 24 hours and at the end of the exposure after approx. 96 hours. Water samples were taken from the middle of the test vessel using a beaker and were transported to the analytical laboratory in glass ampoules, which were rinsed with test solution before they were filled. The transport to the Analytical Laboratory was done on the day of sampling.

Statistics:
No statistical analysis was carried out since no lethality was observed at the highest tested concentration. The median lethal concentration (LC50) was above this value.

	Test substance:	2-Ethylhexylacetate (BASF), purity: 99.3 %
	Reliability:	(1) valid without restrictions
	Flag:	Critical study for SIDS endpoint
	Reference:	BASF AG, Product Safety Department, unpublished study, project no. 12F0674/025053 (to be finalized).
(c)	Value:	9.455 mg/L
	Test substance:	isobutyl isobutyrate (CAS # 97-85-8)
	Remark:	An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
	Reliability:	Score = 2, valid with restrictions, calculated value
	Reference:	ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000.

(d) Value: 3.057 mg/L
Test substance: 2-Ethylhexyl acetate (CAS # 103-09-3)
Remark: An acute fish 96-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability: Score = 2, valid with restrictions, calculated value
Reference: ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

DAPHNIA

(a) Preferred result
Type: static renewal
Test substance: Isobutyl isobutyrate (Eastman IBIB) [CAS No.97-85-8]; 99.6% purity
Species: *Daphnia magna* (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/L
NOEC: 53.7
EC50: 55.8-59.3
Analytical monitoring: yes
Method: OECD 202 and EEC/Annex V C.2
Year: 1995
GLP: yes
Test substance: isobutyl isobutyrate (CAS# 97-85-8)
Method: First-instar Daphnia magna neonates (<24 hours old) were obtained from an in-house culture. Daphnids were cultured in a temperature controlled water bath at 20 degrees C and maintained on a 16 hour light cycle. During holding, daphnids were fed algae and occasionally artifical food. Dilution water was an treated, filtered and aerated water from Lake Ontario. Total hardness and alkalinity were 120 and 91 mg/L as CaCO3, respectively. It was biologically aged by including aquatic organisms in the stored water prior to testing.

The toxicity test was conducted using 250 mL beakers filled to 200 mL with test solution. During testing, vessels were place in a 20±1 deg. C water bath. Light was maintained on a 16h daylight cycle. Daphnids were not fed during the test. No aeration was employed during the test.

Exposure solutions were prepared daily by adding the appropriate amounts of test material to 500-mL volumetric flasks. Aliquots of 200 mL were added to each vessel.

Test concentrations were analyzed daily by gas chromatography (GLC) equipped with a flame ionization detector (GC-FID).

Two replicates of 10 daphnids each were exposed to the control/dilution

water and to each of five concentrations of the TS (95.2, 171.5, 308.6, 555.5, and 1000 mg/L). Renewals were made daily. Numbers of immobilized daphnids and abnormal signs of behavior were recorded at 0, 6, 24, and 48 hours.

Results: Water quality parameters measured during the test included: dissolved oxygen, which ranged from 8.4 to 9.2 mg/L during the test; temperature, which ranged from 20 to 21 deg. C; and pH, which ranged from 6.9 to 9.2.Measured concentrations in the new and 24-h solutions were 53.72-55.82, 70.3-82.8, 109.1-131.35, 227.08-285.2, and 178.15 mg/L (range of two replicates).

Results of the 48-h acute toxicity test, by concentration level tested (for each replicate):
Control: 0, 1 mortality
53.72-55.82 mg/L: 3, 5 mortalities
70.3-82.8 mg/L: 10 (at 24 hours), 10 mortalities
109.31-131.35 mg/L: 10 (at 24 hours), 10 mortalities
227.08-285.2 mg/L: 10 (at 24 hours), 10 mortalities
178.15 mg/L: 10, 10 mortalities (all by 24 hours)

The 48-h LC_{50}s were calculated using the nonlinear interpolation method. Confidence limits were 53.7-82.8 for replicate series A and inestimatable to70.3 mg/L for replicate B.

No sublethal effects were reported.

Reliability: Score = 1, valid without restrictions
Flag: Critical study for SIDS endpoint
Reference: Patterson KM, Hirsch MP. 1995. An Acute Aquatic Effects Test with the Daphnid *Daphnia magna*. Study #EN-431-900945-1, Eastman Kodak Company, Health and Environment Laboratories, Rochester, NY. August 3, 1995.

(b) Data presented because no data for fish and algae are available for isobutyl isobutyrate. Data for the structurally similar 2-ethylhexyl acetate (CAS# 103-09-3) are presented to support the endpoints for isobutyl isobutyrate.

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/L
NOEC: = 15.7
EC50: = 22.9
Analytical monitoring: yes
Method: other: OECD 202 (Part 1)
Year: 2002
GLP: yes
Test substance: 2-ethylhexyl acetate (CAS# 103-09-3)
Method: First-instar Daphnia magna neonates (<24 hours old) were obtained from an in-house culture. Daphnids were cultured in a temperature controlled water bath at 20 degrees C and maintained on a 16 hour light cycle. During holding, daphnids were fed algae and occasionally artifical food. Dilution water was an aged laboratory freshwater. The control / dilution water was a combination of naturally hard well water and well water that was de-mineralized by reverse osmosis yielding the desired range of hardness (total

hardness ranging from 130 to 160 mg/L as CaCO3). It was biologically aged by including aquatic organisms in the tank.

The toxicity test was conducted using 250 mL beakers filled to 200 mL with test solution. During testing, vessels were place in a 20±2 deg. C water bath. Light was maintained on a 16h daylight cycle. Daphnids were not fed during the test. No aeration was employed during the test.

The test employed a water accomodated fraction approach (WAF) in which an 9.0 gram of test material was placed on the surface of water in a 9.5 liter carboy filled with 9 liter dilution water. The solution was stirred for 20 hours with a stir bar creating a vortex <25% solution depth. After stirring the solution was allowed to separate for one hour. The aqueous solution was siphoned from the bottom of the carboy for use. Fresh solutions were prepared daily.

Test concentrations were analyzed daily by gas chromatography (GLC) equipped with a flame ionization detector (GC-FID).

Two replicates of 10 daphnids each were exposed to the control/dilution water and to each of six dilutions of the WAF solution (3.3, 6.5, 13, 25, 50, and 100% WAF). Renewals were made daily. Numbers of immobilized daphnids and abnormal signs of behavior were recorded daily.

Result: Water quality parameters measured during the test included: total hardness, 148 mg/l (as CaCO3); alkalinity, 164 mg/l (as CaCO3); dissolved oxygen ranged from 101 to 117% of saturation during the test; temperature ranged from 19.7 to 22.0 deg. C; and pH ranged from 7.99 to 8.35.

Test solutions were observed to be clear and colorless with no visible particulates, surface film, undissolved test substance, or precipitate.

Measured concentrations in the new solutions ranged from 0.951 to 45.8 mg/L across treatments. Measured concentrations in old solutions (24-h) ranged from <0.688 to 28.1 mg/L. Mean measured concentrations (using ½ the MDL for non-detects) were 0.828, 2.06, 4.12, 7.99, 15.7, and 33.4 mg/L across all treatments.

Results of the 48-h acute toxicity test, by concentration level tested:
Control: No mortality
0.828 mg/L: No mortality
2.06 mg/L: No mortality
4.12 mg/L: No mortality
7.99 mg/L: No mortality
15.7 mg/L: No mortality;
33.4 mg/L: 50% mortality in both replicates at 24 hours, 100% mortality at 48 hours.
No sublethal effects were reported.
The 48-h LC50 was reported as 22.9 mg/l using the Trimmed Spearman-Karber method. Confidence limits were 15.7 to 33.4 mg/L.

Test substance: 2-Ethylhexyl acetate [CAS No.103-09-3]; 99.77% purity, source: Aldrich Chemical Co., Milwaukee, WI, USA.

Reliability: Score = 1, valid without restrictions

Reference: Hahne R. 2002b. Acute Toxicity of the Water Accomodated Fraction (WAF) for 2-Ethylhexyl Acetate to the Water Flea Daphnia magna,

Determined under Static Test Conditions. Report #46956 prepared by ABC Laboratories Inc., Columbia Missouri. May 02, 2002.

(c)
Value:	27.566 mg/L
Test substance	Isobutyl isobutyrate (CAS # 97-85-8)
Remark:	An acute daphnid 48-h LC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability:	(2) valid with restrictions, calculated value
Reference:	ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000.

(d)
Value:	3.571 g/L
Test substance:	2-ethylhexyl acetate (CAS# 103-09-3)
Remark:	An acute daphnid 48-h LC50 was calculated using ECOSAR, from the US EPA. The SAR for esters was used. The structure was determined using the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability:	Score = 2, valid with restrictions, calculated value.
Reference:	ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000.

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

(a) No ecotoxicity studies with fish or algae are available for isobutyl isobutyrate. Data for the structurally similar 2-ethylhexyl acetate (CAS# 103-09-3) are presented to support the endpoints for isobutyl isobutyrate.

Preferred result

Species:	*Selenastrum capricornutum* (Freshwater green algae)
Test substance:	2-Ethylhexyl acetate [CAS No.103-09-3]; 99.77% purity, source: Aldrich Chemical Co., Milwaukee, WI, USA.
Endpoint:	growth inhibition (based on area under growth curve), cell counts
Exposure period:	72 hour(s)
Units:	mg/L
Limit test:	no
EC50:	>21.9
NOEC:	10.3
LOEC:	21.9
Analytical monitoring:	yes
Method:	OECD Guideline 201
GLP:	yes
Test substance:	2-Ethylhexyl acetate (CAS# 103-09-3)
Method:	The green alga *Selenastrum capricornutum* used was maintained in the laboratory and originated from the Univ. Texas Culture Collection (UTEX #1648), Austin, TX. Cultures were maintained in synthetic algal nutrient medium in flasks under continuous cool-white fluorescent illumination of ~8000 lumens/m2 at 24±2°C and continuously shaken at 100 oscillations/minute. Test medium was prepared by adding requisite amounts of each of the macro- and micro-nutrients into laboratory-grade water. After pH adjustment to 7.5±0.1 S.U., the media was filtered using a 0.45 micrometer porosity filter and refrigerated.

The test employed a water accomodated fraction approach (WAF) in which an 3.0 gram of test material was placed on the surface of water in a 4 liter carboy filled with 3 liter dilution water. The solution was stirred for 20 hours with a stir bar creating a vortex <25% solution depth. After stirring the solution was allowed to separate for one hour. The aqueous solution was siphoned from the bottom of the carboy for use and was as the 100% WAF.

Test vessels were sterilized 250-mL flasks fitted with foam stoppers. Besides six control replicates, three replicates were prepared for each test concentration. Replicates contained 100 mL test solution. Nominal test concentrations of 6.5, 13, 25, 50, and 100% WAF were chosen based on the results of two range finding tests.

Each flask (except for a fourth flask of the lowest concentration used for 72-h chemical analysis) was inoculated with 1 mL of the algae containing approximately 1.0 E+6 cells/mL, resulting in an initial cell density of approximately 1.0 E+4 cells/mL. Flasks were kept under the same conditions as the stock cultures. Cell counts were performed using a light microscope and a hemacytometer every 24 hours in all flasks. Results were expressed as cells/mL.

Temperature and pH was measured in each test vessel and control at test initiation and in one replicate of the control and each treatment at 72 hours.

Samples of the initial test solutions were analyzed for TS concentration. A fourth replicate at the lowest concentration was analyzed at 72 hours. Chemical analysis was performed using gas chromatography (GC) equipped with a Flame Ionization Detector (FID).

Final samples were prepared by extracting a 10-mL sample twice with toluene and shaken for two minutes. Samples were vialed and immediately analyzed.

EC and NOEC values were calculated based on area under the growth curve and growth rate versus mean measured concentration. Shapiro-Wilk's test and Levene's test were conducted to test for normality and homogeneity of variances. A one-way ANOVA and a Dunnett's comparison to the control was conducted for each time point to determine the NOECs.

Results:

Temperature ranged from 23.5 to 25.0°C at 0 and 72 hours. The pH values ranged from 7.40 to 7.57 S.U. at test initiation across all test vessels and pH ranged from 8.97 to 9.26 S.U. at 72 hours across all vessels. Although the pH increased by more than 1 pH unit in some flasks, the integrity of the test was deemed unaffected, since the control performance was unaffected (control pH at 72 hours was 9.03 S.U.)

Measured concentrations on day 0 ranged from 2.49 to 43.4 mg/L across treatments (6.5 to 100% WAF). Measured concentrations in day 3 solutions were all <0.688. Mean measured concentrations (using ½ the MDL for non-detects) were 1.42, 2.70, 5.27, 10.3, and 21.9 mg/L across all treatments.

At 72 hours, the control had 116 E+4 cells/mL, an increase of 116 times that at test initiation indicating an acceptable test. The percent change in cell density ranged from -3% in the 25% and 100% WAF treatments to +14% in

the 13% WAF treatment. At 72 hours, no significant reduction in algal growth was fund in treatments #50% WAF as measured by area under the growth curve and #100% WAF as measured by growth rate.

72-h EC50 = >21.9 mg/L (based on area under the growth curve and growth rate)
72-h NOEC = 10.3 mg/L, 72-h LOEC = 21.9 mg/L

Reliability: Score = 1,valid without restrictions
Flag: Critical study for SIDS endpoint
Reference: Hicks SL. 2002. Toxicity of 2-Ethylhexyl Acetate to the Unicellular Green Alga, Selenastrum capricornutum. Report #46957 prepared by ABC Laboratories Inc., Columbia Missouri. May 01, 2002.

(b) Value: 0.771 mg/L
Test substance: Isobutyl isobutyrate (CAS# 97-85-8)
Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability: (2) valid with restrictions, calculated value
Reference: ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000.

(c) Value: 0.26 mg/L
Remark: An acute algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Test substance: 2-Ethylhexyl acetate [CAS No.103-09-3]
Reliability: (2) valid with restrictions, calculated value
Reference: ECOSAR model (v.0.99g). EPISUITE v. 3.11. U.S. Environmental Protection Agency, 2000.

4.4 TOXICITY TO BACTERIA

No data available

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

No data available

4.5.1 CHRONIC TOXICITY TO FISH

No data available

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

No data available

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

No data available

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data available

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

No data available

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

No data available

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

No data available

4.8 BIOTRANSFORMATION AND KINETICS

No data available

4.9 ADDITIONAL REMARKS

No additional remarks

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Isobutyl isobutyrate (IBIB) is rapidly broken down to form equal amounts of isobutyric acid and isobutanol. The resulting isobutanol is further broken down to ultimately form isobutyric acid. In order to support the limited acute oral toxicity information on IBIB, acute oral toxicity data for the two metabolites (isobutanol and isobutyric acid) have been added to this section.

(a) Preferred result

Type:	LD_{50}
Species:	rat
Value:	>6400 mg/kg
Method:	The test material was administered undiluted to seven animals at dose levels ranging from 200 to 12800 mg/kg. One rat was used at each dose level. The animals were observed for 14 days following dosing and no necropsies were performed.
Year:	1956
GLP:	no
Test substance:	isobutyl isobutyrate
Remark:	Weakness, and ataxia were observed in the animal receiving 12800 mg/kg. The time of death was the 2nd day following dosing.
Reliability:	Score = 2, reliable with restrictions due to study design
Flag:	Critical study for SIDS endpoint
Reference:	Laboratory of Industrial Medicine, Eastman Kodak Co., 1956

(b)

Type:	LD_{50}
Species:	mouse
Value:	>6400 mg/kg
Method:	The test material was administered undiluted to seven animals at dose levels ranging from 1600 to 25600 mg/kg. One mouse was used at each dose level. The animals were observed for 14 days following dosing and no necropsies were performed.
Year:	1956
GLP:	no
Test substance:	isobutyl isobutyrate
Remark:	Weakness, and ataxia were observed in the animal receiving 12800 mg/kg. The time of death was within 3 hours following dosing.
Reliability:	Score = 2, reliable with restrictions due to study design
Reference:	Laboratory of Industrial Medicine, Eastman Kodak Co., 1956

(c)

Type:	LD_{50}
Species:	rat
Value:	> 2830 mg/kg bw (males) 3350 (2860 to 3920) mg/kg bw (females)
Method:	EPA (TSCA) Health Effects Testing Guidelines 40 CFR Part 798 (Subpart B, Section 798.1175:acute oral toxicity; and 1987 OECD Guidelines for Testing of Chemicals (Section 4: Health Effects; 401:acute oral toxicity). Rat (Harlan Sprague Dawley) body weights were within +/- 20% of the group mean for each sex. The body weight range on the day of dosing was 281 to 292 g for males and 210 to 259 g for females (including those used for preliminary testing). A total of 3 male and 20 female rats were used for the definitive peroral test. An additional 2 female rats were used for preliminary testing. The animals were acclimated for at least 5 days before

dosing. Detailed clinical observations were conducted twice, at the time of receipt and during animal identification and/or dosing. Cage-side observations and mortality checks were conducted at least once daily. Animals considered unacceptable for the study, based on the clinical signs were rejected for use on this study. Each dosing mixture was prepared just prior to administration by diluting the appropriate amount of isobutanol with 0.25% w/v aqueous methyl cellulose solution. All resulting emulsions were mixed for approximately 15 to 30 minutes on a magnetic stirrer. Doses were administered by stomach intubation through a commercial 16-gauge (3-inch) ball-end stainless steel needle attached to a disposable syringe. The exact amounts of test substance and emulsion given to each rat were recorded on the raw data form. The rats were fasted overnight before dosing. Five female rats were included on each of several dose levels in order to determine an LD_{50}. Three male rats were included on an intermediate dose level for comparison. An additional 2 female' rats were used for preliminary peroral toxicity testing. For individual animals, the dosing volume was adjusted according to body weight. Dosed rats were observed frequently for signs of toxicity on the first day of the test and twice a day thereafter (except on weekends or holidays when they were examined for death alone). Weights were recorded on the day of dosing and at 7 and 14 days after dosing or at death. After 14 days, all survivors were sacrificed by CO_2 overdose. Necropsies were performed on all animals that died or were sacrificed. Unless tissues were judged to be excessively autolyzed, the following tissues were collected from selected animals and retained in 10% neutral buffered formalin: kidneys, urinary bladder, liver, sciatic nerve, stomach, intestines and spleen. Lungs were also saved because of possible lung damage, based on clinical signs. An LD_{50} was calculated for female rats, based on the 14-day observation period. It was calculated by the moving average method. An estimate of the slope was made by the formula developed by Weil. During the acute peroral toxicity test, several animals (including survivors) had varying amounts of blood present in the urine. Therefore, histology evaluations were performed on all saved kidney and urinary bladder tissues. One female rat appeared to be pregnant at necropsy and the uterus was saved in order to verify this condition (since the animals are ordered to be non-pregnant).

Year: 1993

GLP: Yes

Test substance: Isobutanol (99.9% purity by capillary GC, GC/MS and NMR used to confirm identity)

Remark: In preliminary testing, 1 female rat was dosed with 2000 mg/kg of isobutano1 and 1 female rat was dosed with 8000 mg/kg (20% w/v emulsions in 0.25% aqueous methyl cellulose solution). The rat receiving 8000 mg/kg died. In the definitive test, the peroral LD_{50} for female rats dosed with the test substance (emulsions in 0.25% aqueous methyl cellulose solution) was 3350 mg/kg.

None of 3 male rats died after receiving peroral doses of 2830 mg/kg of isobutano1 (a comparison dose that produced 0 of 5 female deaths), although signs were apparent. Signs of toxicity included sluggishness, unsteady gait, lacrimation, piloerection, slow breathing, prostration and a trace to large amount of blood in urine (positive by HEMASTIX. Reagent Strips). Several females exhibited a slight weight loss within 7 to 14 days. Deaths occurred within 2 hours to 1 day. Survivors recovered within 0.5 hour to 6 days. Necropsy of animals that died revealed discolored and/or mottled lungs (bright to dark red), tan to dark maroon and/or mottled livers (in 2), discolored stomachs (gray and/or yellow), 1 liquid-filled stomach,

dark red and/or gray areas on the intestines, red to brown kidneys (in 1) and a large amount of blood in the urine of 1 (positive by HEMASTIX. Reagent Strips). There were no gross lesions apparent in any survivor at necropsy. One female survivor dosed with 2830 mg/kg of isobutanol appeared pregnant at necropsy (determined to be a pseudopregnancy during microscopic valuation). The kidneys and urinary bladders from 1 or 2 rats from each dose group (except 1000 mg/kg) were saved and examined microscopically (see Appendix 2). The only kidney lesions evident were single instances of tubular proteinosis, tubular basophilia, mineralization and congestion, which were not considered to be attributable to the test substance. There were no lesions observed in the urinary bladders. In the uterus of the 1 female rat (2830 mg/kg) that appeared pregnant at necropsy, deciduoma of pseudopregnancy were apparent. This condition is somewhat unusual for animals of this age group. Subsequent investigations revealed that the female rats ordered for this study had undergone vaginal swabbing on the day of shipment at the animal supplier. This female animal (and one other from the acute inhalation study) had pseudopregnancy due to cervical stimulation from the vaginal swabbing procedure. The male rat oral (fasted) LD_{50} was > 2830 mg/kg bw; 0 of 3 died. The female rat oral (fasted) LD_{50} was 3350 (2860 to 3920) mg/kg bw.
Microscopic kidney lesions were evident but probably not related to treatment.

Reliability: Score = 1, reliable without restrictions. GLP guideline study for Isobutanol
Reference: Christopher, S.M. November 30, 1993. "Isobutanol: Acute toxicity and irritancy testing using the rat (peroral and inhalation toxicity) and the rabbit (cutaneous and ocular tests)". Bushy Run Research Center, Union Carbide Corp. Lab. Proj. ID 92U1166

(d) Type: LD_{50}
Species: rat
Value: >500 mg/kg
Method: Ten male albino rats (Charles River CD strain) were fasted 18 hours prior to receiving 500 mg/kg of isobutyric acid as a 2% suspension in corn oil. Clinical signs were observed on the day of dosing and daily during the 14-day observation period. The initial and terminal body weights were determined on the test animals. A gross autopsy was not performed.
Year: 1979
GLP: no data
Test substance: isobutyric acid
Remark: No signs of intoxication were observed and all animals gained weight normally during the 14 day observation period. This study was conducted in support of the use of isobutyric acid as a fumigant in dry corn to prevent growth of mold and fungus.
Reliability: Score = 2, reliable with restrictions; accepted scientific standard methods – For Isobutyric acid
Reference: Report No. A79-146. May 25, 1979. Toxicity Data Report. Animal Industry R/D, Agricultural Division, American Cyanamid Co. P.O. Box 400, Princeton, NJ 08540

5.1.2 ACUTE INHALATION TOXICITY

(a) Preferred result
Type: LC_{66} and LC_0

	Species:	rat
	Value:	LC_{66} = 5423 ppm, LC_0 = 658 ppm
	Method:	Groups of three rats were exposed to an atmospheres containing aerosolised isobutyl acetate at a concentration of either 5423 or 658 ppm for 6 hours. Exposure values were based on nominal concentrations.
	Year:	1956
	GLP:	no
	Test substance:	isobutyl isobutyrate
	Remark:	No effects were noted in the rats exposed to 658 ppm for 6 hours. Clinical signs noted in the 5423 ppm group included prostration and narcosis. Deaths occurred during exposure. All animals that survived the exposure gained weight at the end of the 14 day observation period.
	Reliability:	Score = 2, reliable with restrictions; accepted scientific methodology
	Flag:	Critical study for SIDS endpoint
	Reference:	Laboratory of Industrial Medicine, Eastman Kodak Co., 1956
(b)	Type:	Acute inhalation study with neurobehavioral battery
	Species:	Male and female SD rats
	Exposure levels:	0, 1500, 3000, and 6000 ppm (0, 4545, 9090, 18,180 mg/m^3)
	Method:	Male and females rats (10/sex/concentration) were exposed to isobutanol for 6 hours, immediately followed by a motor activity determination and a functional observational battery (FOB). All of the rats on study were subdivided into four FOB assessment groups and exposed (and data collected) on different days in order to obtain timelier FOB assessments. Body weight data was collected on Day 1 (pre-exposure), 7, and 14. During exposure assessments were limited to a crude startle response reflex determination for the animals visible thru the exposure chamber windows. The stimulus startle response was initiated by sharply striking an object against the stainless steel exterior wall of the chamber. Post-exposure motor activity (60 minutes) and FOB tests were conducted pre-test (1-2 weeks prior to exposure), immediately following exposure (Day 1) and seven and fourteen days after the exposure. An additional motor activity test was conducted on Day 2. FOB assessments were conducted approximately 10-30 minutes after the motor activity test ended. An automated apparatus was used to conduct motor activity tests while trained observers blind to the test status of the animals conducted the FOB tests. A two-way ANCOVA and Duncan's multiple comparison test was used to determine statistical significance. The FOB evaluation was similar to methods published by Mosher (1991).
	Year:	2002
	GLP:	Yes
	Test substance:	isobutanol (99.9% purity)
	Remark:	Exposure concentrations were within 10% of target. No exposure related differences were noted between the control and exposed groups. Hypoactivity and diminished response to a startle reflex was observed during exposure for the 3000 and 6000 ppm exposures. Decreases in motor activity were noted post-exposure in the 6000 ppm groups but not the 3000 or 1500 ppm groups. No effect on motor activity was detected at the 7 and 14 day time points. No exposure-related effects were noted in the FOB assessment.
	Reliability:	Score = 1, reliable without restrictions; GLP guideline study
	Reference:	Li, A.A., Kaempfe, T.A., O'Donnell, P.E., Smolboski, D. 1994. Acute Neurotoxicity Study of Isobutanol in Sprague-Dawley Rats. Monsanto Project No. EHL 94009 and Union Carbide Laboratory Project No. 37-AEG-131.

5.1.3 ACUTE DERMAL TOXICITY

(a) Preferred result

Type:	LD_{50}
Species:	guinea pig
Value:	>10 ml/kg
Method:	According to the 24 hour cuff method (Draize, et al.,)
Year	1956
GLP:	no
Test substance:	isobutyl isobutyrate
Remark:	Contact period = 24 hours, observation period = 14 days. Based on a lack of weight gain in the animal receiving 10 ml/kg for 24 hours under an occlusive cuff, isobutyl isobutyrate can be absorbed thru the skin and cause toxicity.
Reliability:	Score = 2, accepted scientific methodology
Flag:	Critical study for SIDS endpoint
Reference:	Laboratory of Industrial Medicine, Eastman Kodak Co., 1956

5.2.1 SKIN IRRITATION/CORROSION

(a) Preferred result

Species:	guinea pig
Result:	slight irritant
Classification:	
Method	A gauze pad soaked with undiluted isobutyl isobutyrate was held in contact with the shaved skin of a guinea pig under an occlusive wrap for 24 hours. The animals were observed for 14 days after exposure.
Year:	1978
GLP:	no
Test substance:	isobutyl isobutyrate
Remark:	Slight irritation indicates some redness and scaling of the skin.
Reliability:	Score = 2, accepted scientific methodology
Reference:	Laboratory of Industrial Medicine, Eastman Kodak Co., 1956

5.2.2 EYE IRRITATION/CORROSION

No data available

5.3 SKIN SENSITISATION

No data available

5.4 REPEATED DOSE TOXICITY

Isobutyl isobutyrate (IBIB) is rapidly broken down to form equal amounts of isobutyric acid and isobutanol. In order to compare the toxicity information on IBIB with isobutanol, a repeated-dose oral toxicity study (also conducted by gavage) for isobutanol has been added to this section. There are no repeated-dose toxicity studies for isobutyric acid.

(a) Preferred result

Species:	rat
Strain:	Wistar
Sex:	male and female

	Route of Admin:	oral gavage as a solution in corn oil
	Exposure Period:	18 weeks
	Freq. of Treatment:	7 days/week for 18 weeks
	Post Exposure Observation Period:	none
	Doses:	0, 10, 100, and 1000 mg/kg
	Control Group:	yes
	NOAEL:	1000 mg/kg
	LOAEL:	NA
	Method:	The study consisted of male and female animals (15/sex/group) receiving 0, 10, 100, or 1000 mg/kg isobutyl isobutyrate by oral gavage for 18 weeks. The dose volume was 5 ml/kg with the control animals receiving corn oil alone. In addition, groups of five rats of each sex were given the same doses for either 2 or 6 weeks. The animals were weighed on Day 1, 2, 6 and weekly thereafter. Feed and water consumption were determined on a cage basis (5/cage by sex). After the final dose, the animals were fasted for 24 hours and killed under barbiturate anesthesia. Hematology parameters were collected at 3, 6, and 18 weeks inot the study. A gross necropsy was performed and brain, heart, liver, spleen, kidney, adrenals, stomach, small intestine, caecum, gonads, pituitary, and thyroid(s) were weighed. Typical organs were collected, fixed in formalin, and processed for histological exam. Urine was collected during week 2, 6, and 18 of treatment and examined. Kidney function tests were also performed during week 6 and 18.
	Year:	1977
	GLP:	no
	Test substance:	isobutyl isobutyrate (>98% pure).
	Remark:	This study was performed due to the food additive use.
	Results:	No deaths or abnormal behaviour was noted during the study. No differences in body weight, feed or water consumption, or histological findings were noted. The only change in haematology parameters was a decrease in hemoglobin concentration in the male 100 and 1000 mg/kg bw/day groups after two weeks of exposure. These changes were not accompanied by changes in other red blood cell parameters in these groups, and the female animals were not affected. In addition, similar findings were not observed at the 6 and 18-week haematology determinations. Kidney function tests and urine examination was normal. The only change in organ weights was an increase (14%) in relative spleen weight (when corrected for body weights) in the 1000 mg/kg bw/day male group. The mean terminal body weights of animals in this group were decreased by 7% compared to the control group (not statistically significant but may explain the increased relative spleen weight). Due to the fact that there were no histological changes in this or any other organ, this finding was considered spurious.
	Reliability:	Score = 2, reliable with restrictions; accepted scientific methodology
	Flag:	Critical study for SIDS endpoint
	Reference:	Drake, J. J-P., K.R. Butterworth, I.F. Gaunt, and P. Grasso. 1978. Short-Term Toxicity Study of Isobutyl Isobutyrate in Rats. Food Cosmetics Toxicology, 16:337-342.
(b)	Species:	rat
	Strain:	CD
	Sex:	male and female
	Route of Admin:	gavage
	Exposure Period:	90 days
	Freq. of Treatment:	daily

Post Exposure Observation Period:	N/A
Doses:	0, 100, 316, or 1000 mg/kg/day
Control Group:	yes
NOAEL:	316 mg/kg
LOAEL:	1000 mg/kg
Method:	Four groups of male and female rats (30/sex/group) were dosed daily by gavage with 0, 100, 316 or 1000 mg/kg/day of isobutanol for either 4 weeks (interim sacrifice; 10/sex/group) or 13 weeks (remaining animals). Dosing solutions of isobutanol in deionized water were used and 10 mL/kg was the constant dosing volume. Body weights and feed consumption were recorded weekly. Clinical signs were recorded daily. Blood and urine were collected for clinical pathology at pre-dose (10 sentinel animals), and at the 4 and 13-week necropsies. Organ weights and results of gross pathology exams were recorded at both the 4 and 13-week necropsies. Histopathological examinations of tissues from the control and 1000 mg/kg groups were conducted as well as examination of hearts, livers, and kidneys from the 100 and 316 mg/kg dose groups.
Year:	1987
GLP:	yes
Test substance:	isobutanol (purity 99.9%)
Remark:	Analysis of dosing solutions confirmed concentrations and stability. The difference between the presence or absence of acute clinical signs of toxicity (ataxia, hypoactivity) following oral administration between the isobutanol and isobutyl isobutyrate studies is most probably due to the vehicle used. The isobutanol study uses distilled water, a vehicle that would allow rapid absorption of the alcohol while the limited solubility of isobutyl isobutyrate in water necessitated the use of a corn oil vehicle. The corn oil vehicle would allow for a slower absorption of the test article and hence, the lack of acute signs of intoxication.
Results:	Treatment-related clinical signs noted in the 1000 mg/kg dose group included hypoactivity, ataxia, salivation, labored respiration, rales, prostration, hypothermia, and emaciation. Hypoactivity and ataxia were the most common clinical signs and these resolved primarily after week 4. There were no compound related clinical signs in the 100 or 316 mg/kg dose groups. The mortality rate was 1/60, 1/60, 2/60, and 11/60 for the control, 100, 316, and 1000 mg/kg groups, respectively. The only difference in body weights, body weight gain, or feed consumption was during weeks 1 and 2 of the study and were restricted to the 1000 mg/kg/day dose group. In addition, there were no dose-related differences observed in organ weights, gross pathology or histopathological examination. The mortality observed in the different dose groups was due to gavage errors, and was not due to compound administration.
Reliability:	Score = 2, reliable with restrictions; standard method with restrictions; conducted with isobutanol
Reference:	"Rat Oral Subchronic Toxicity Study Final Report. Compound: Isobutyl Alcohol." Toxicity Research Laboratories, Ltd. Muskegon, MI. TRL Study #032-002 dated 1987.

5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL IN VITRO TEST

(a) Preferred value

Type:	Ames test with additional E. coli strain
System of Testing:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, Escherichia coli strain WP2uvrA-
Concentration:	up to 5000 ug/plate
Metabolic Activation:	with and without
Result:	Isobutyl isobutyrate was non-mutagenic when tested to a maximum concentration of 5000 micrograms/plate, using the plate incorporation protocol. At least five doses were tested in triplicate, without metabolic activation, and with 10% liver S-9 from rat. Replicate testes were performed after the initial trial to confirm these results.
Method:	Isobutyl isobutyrate was tested as a coded chemical using the plate incorporation method using S. typhimurium tester strains, TA98, TA100, TA1535, and TA1537 and E. coli strain WP2uvrA- in the presence and absence of phenobarbitone/β-naphthoflavone-induced liver S9 from male Sprague Dawley rats. Test concentrations of isobutyl isobutyrate (up to 5000 ug/plate) were prepared using dimethyl sulphoxide as the solvent; a maximum of 0.1 ml solvent was added to each plate. Each dose was tested in triplicate without activation, and with 10% rat liver S-9. Concurrent positive and solvent controls were run with each trial. Repeat experiments were performed following the initial trial. A material was considered mutagenic if it produced a reproducible, dose-related increase in revertants over the solvent control, under a single metabolic activation condition, in replicate trials. A material was considered questionable if the positive response was elicited at only one concentration, or if the response could not be reproduced. A chemical was designated as non-mutagenic only after it was tested without metabolic activation, and with 10% rat S-9.
Year:	2003
GLP:	Yes
Test substance:	isobutyl isobutyrate
Purity:	>99%
Reliability:	Score = 1, reliable without restrictions; guideline study
Flag:	Critical study for SIDS endpoint
Reference:	SafePharm communication to Karen Ruble, Eastman Chemical Company, December 4, 2003.

B. NON-BACTERIAL IN VITRO TEST

5.6 GENETIC TOXICITY IN VIVO

Data from isobutanol in vivo genetic toxicity studies have been included in this section. Data from isobutanol is useful when assessing the hazard associated with isobutyl isobutyrate exposure due to the rapid and complete hydrolysis of isobutyl isobutyrate to isobutanol in vivo. There are no in vivo genetic toxicity studies conducted with isobutyric acid. The toxicokinetics of the metabolism is documented and explained further in the Toxicokinetics Section (5.10.B).

(a) Preferred result

Test substance:	isobutanol
Test species:	Mouse/NMRI (male and female)

Test method:	OECD No. 474 (Proposal for updating, ENV/EPOC (96)4) EPA/TSCA 789.5395 (August 1997) EEC Directive 92/69, B 12 (December 1992)
GLP:	yes
Test results:	Oral gavage dose of 500, 1,000 or 2,000 mg/kg of isobutanol did not have any chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis.
Lowest dose producing toxicity:	1000 mg/kg
Effect on Mitotic Indexor P/N Ratio:	None
Genotoxic effects:	negative
Comments:	Both of the positive control chemicals, i.e. cyclophosphamide for clastogenicity and vincristine for spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei.
Reliability:	Score = 1, reliable without restrictions; GLP guideline study
Flag:	Critical study for SIDS endpoint
Reference:	Engelhardt, D., and Hoffmann, H.D. Cytogenetic Study In Vivo with Isobutanol in the Mouse Micronucleus Test - Single Oral Administration. (2000) Project No. 26M0243/994085, Department of Toxicology, BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, FRG.

5.7 CARCINOGENICITY

No data available

5.8 TOXICITY TO REPRODUCTION

Data from a two-generation reproductive toxicity study with isobutanol has been included in this section. Data from isobutanol is useful when assessing the hazard associated with isobutyl isobutyrate exposure due to the rapid and complete hydrolysis of isobutyl isobutyrate to isobutanol in vivo. There are no reproductive toxicity studies conducted with isobutyric acid. The toxicokinetics of the metabolism is documented and explained further in the Toxicokinetics Section (5.10.B).

(a) Preferred value

Type:	Two generation study
Species/strain:	Rat/Sprague-Dawley
Sex:	Male and Female
Route of Adm.:	inhalation
Method:	Conducted according to US EPA Health Effects Test Guidelines OPPTS 870.3800, Reproduction and Fertility Effects, August 1998. Briefly, groups of male and female rats (30/sex/group) were exposed to 0, 500, 1000, or 2500 ppm isobutanol for six hours/day, seven days/week for ten weeks prior to mating. Exposures continued in the male animals until sacrifice. The female animals were exposed through gestation day 20, with exposure reinitiated on lactation day 5 and continued through lactation day 28. The F1 pups were weaned on postnatal day 29 and those chosen to represent the next generation started direct inhalation exposures on postnatal day 29. These F1 male and female animals (30/sex/group) were exposed for ten weeks prior to mating. The F1 males continued exposure until sacrifice. The F1 female animals were exposed through gestation day 20, with

	exposure reinitiated on lactation day 5 and continued through lactation day 21. Body weight, feed consumption, exposure parameters, necropsy endpoints, and reproductive and developmental endpoints were collected according to the test guideline.
Exposure period:	6 hours/day
Freq. of treatment:	7 days/week prior to mating, during mating and gestation; treatment was suspended during lactation days 0-4 and re-initiated on lactation day 5.
Premating exposure period:	10 weeks
Exposure conc.:	0, 500, 1000 and 2500 ppm
Control group:	Concurrent
NOAEL Parental:	2500 ppm
NOAEL F1 Offspring:	2500 ppm
NOAEL F2 Offspring:	2500 ppm
Results:	Exposure to isobutanol concentrations up to 2500 ppm did not cause any parental systemic, reproductive, or neonatal toxicity when administered for two generations via whole body exposure.
GLP:	yes
Test substance:	isobutanol (>99.9% purity)
Remarks:	The highest exposure concentration was chosen based upon decreases in reaction to an external stimuli reported in a previous neurotoxicity study (Le, et al., 2001). However, the animals exposed to 2500 ppm in this study did not demonstrate decreases in response to external stimuli as was previously reported.
Reliability:	Score =1: reliable without restrictions; GLP guideline study
Flag:	Critical study for SIDS endpoint
Reference:	"An inhalation two-generation reproductive toxicity study of isobutanol in rats." WIL Research Laboratory Study Number WIL-186013, WIL Research Laboratories, Inc., 1407 George Rd, Ashland, OH 44805-9281, sponsored by the Oxo-Process Panel of the American Chemistry Council, 1300 Wilson Boulevard, Arlington VA 22209.2003

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Data from isobutanol developmental toxicity studies have been included in this section. Data from isobutanol is useful when assessing the hazard associated with isobutyl isobutyrate exposure due to the rapid and complete hydrolysis of isobutyl isobutyrate to isobutanol in vivo. There are no developmental toxicity studies conducted with isobutyric acid. The toxicokinetics of the metabolism is documented and explained further in the Toxicokinetics Section (5.10.B).

(a) Preferred value

Species:	rat
Strain:	Wistar
Sex:	female
Route of Admin:	inhalation
Exposure Period:	Day 6 through 15 of gestation
Frequency of Treatment:	Daily for 6 hours/day
Duration of Test:	10 treatment days/animal
Exposure Conc.:	0, 0.5, 2.5, or 10.0 mg/L
Control Group:	yes, concurrent no treatment

LOAEL (Maternal Toxicity):	10.0 mg/L
NOAEL (Teratogenicity):	10.0 mg/L
Method:	Pregnant rats were exposed to isobutanol by whole body exposure from gestation day 6 through 15. Body weights, feed consumption, and clinical sign data were collected throughout the study. Chamber concentrations (actual and nominal), temperature, and absolute and relative humidity values were collected.
Year:	1990
GLP:	yes
Test substance:	isobutanol (purity >99.8%)
Result:	No treatment related effects on either the dams or the offspring were observed. Therefore, under the conditions of this study, 10 mg/l was considered a No-Observed-Effect Level for both maternal and fetal outcomes.
Reliability:	Score = 1, GLP guideline study
Flag:	Critical study for SIDS endpoint
Reference	Klimisch, H.-J. 1990. Prenatal Toxicity of 2-Methyl-1-Propanol in Rats After Inhalation. Project No. 37R0057/88047. BASF Department of Toxicology, BASF Corp. 6700 Ludwigshafen, West Germany.

(b) Species: rabbit

Strain:	Himalayan
Sex:	Female
Route of Admin.:	Inhalation
Exposure Period:	Day 7-19 of gestation
Freq. of Treatment:	6 h/day
Duration of Test:	Up to Day 29 post-implantation
Exposure Concentrations:	0.5; 2.51; 10 mg/L
Control Group:	Yes
NOAEL Maternal Toxicity:	2.51 mg/L
NOAEL Developmental Toxicity:	10 mg/L
Method:	OECD Guideline 414 "Teratogenicity"
Year:	1990
GLP:	Yes
Test substance:	Isobutanol purity >99.8%
Result:	Each control and study group contained 15 pregnant females. A slight (non-significant) retardation in body weight was observed in rabbits of the high-dose group throughout the exposure period. Otherwise, no compound-related effects indicative of maternal toxicity were found. Significantly increased incidences of intraventricular foramen/septum membranaceum (cardiac septal defects) were found for the high-dose group; this finding was not considered to be of biological significance, because by comparison with historical control data, the incidences were found to lie fully within the range of biological variation. Substance related effects on the offspring, indicative of embryo-/fetotoxicity or teratogenicity, were not observed.
Reliability:	Score = 1, reliable without restrictions; GLP guideline study
Reference:	BASF AG, Department of Toxicology: "Prenatal Toxicity of 2-Methyl-1-propanol in Rabbits After Inhalation", BG No.96, Project No.

90R0057/88048, 12.14.1990, conducted under the auspices of the BG Chemie, Heidelberg, (1990); Klimisch H.-J. and Hellwig J.: Fund. Appl. Toxicol., 27, 77-89, (1995).

5.10 OTHER RELEVANT INFORMATION

A. SPECIFIC TOXICITIES

No data available

B. TOXICODYNAMICS, TOXICOKINETICS

(a) Preferred value

Species:	rat
Strain:	Sprague-Dawley
Sex:	male
Route of Admin:	intravenous
Exposure Period:	Bolus injection into indwelling catheter
Freq. of Treatment:	Single
Duration of Test:	Intravenous blood sampling occurred from immediately post dosing until 240 seconds (4 minutes) after bolus injection
Exposure Conc.:	0.125 mmol/Kg
Control Group:	no, used pre-injection samples as background controls
Method:	Preliminary studies were conducted to select dose levels, dose formulations, and sampling times for the definitive studies. Isobutyl isobutyrate in saline with 1% Tween 20 was administered individually to seven animals via an indwelling femoral vein catheter. Serial blood samples were collected from an indwelling jugular vein catheter and immediately deproteinized to halt enzymatic activity. All catheters were made of Teflon as the test material adhered to the other types of plastic catheter materials. Extensive methods development was conducted to insure the test material was delivered into the systemic circulation and that the sampling times would provide useful metabolism/toxicokinetic data. The entire sampling period lasted only 240 seconds. Concentrations of isobutyl isobutyrate as well as down stream metabolites (isobutanol and isobutyric acid) were assayed by an internal standard GC-MS selected ion monitoring method.
Year:	2003
GLP:	yes
Test substance:	isobutyl isobutyrate purity > 99%
Result:	Following intravenous administration of isobutyl isobutyrate, blood collection were done as fast as possible due to the rapid metabolism of the test article. Analysis of these blood samples demonstrated a extremely rapid hydrolysis of isobutyl isobutyrate to form isobutanol and isobutyric acid. The estimated $T_{1/2}$ (from a simple one-compartment model with bolus input and first-order output) was 11.1 seconds. Peak isobutyl isobutyrate levels were found in the less than 15 seconds sampling time period with mean values of 1045 micromolar. The isobutyl isobutyrate levels decreased very quickly (by 46 seconds, they were at 43 micromolar) and could not be detected 166 seconds after dose administration. Isobutanol levels increased to the 78-220 micromolar range within the 15 seconds required for the first sample and stayed in this range until the end of sampling at 240 seconds. Isobutyric acid levels increased up to 304 micromolar within the 31-45 seconds time point and remained above 100 micromolar until 196 seconds.

Isobutyric acids were consistently higher than the isobutanol levels suggesting formation of isobutyric acid from the further metabolism of isobutanol. This study demonstrates a extremely rapid hydrolysis of isobutyl isobutyrate, with a half-life measured in seconds. It also demonstrates the rapid appearance of the down stream metabolites, isobutanol and isobutyric acid.

Isobutyl isobutyrate, isobutanol and isobutyric acid blood levels Found following isobutyl isobutyrate intravenous injection.

Sampling Time (seconds)	Isobutyl isobutyrate*	Isobutanol	Isobutyric acid*
0	Nd**	Nd	Nd
<15	1045	103	129
16-30	405	209	268
31-45	115	218	304
46-60	43	196	295
61-75	18	163	252
76-90	12	153	225
91-105	8	135	188
106-120	6	115	161
121-135	6	128	162
136-150	3	97	126
151-165	6	103	126
166-180	Nd	98	122
181-195	Nd	87	107
196-210	Nd	78	95
211-225	Nd	82	98
226-240	Nd	96	107

*mean μM whole blood, **Nd = not detected

Reliability: score = 2, reliable with restrictions; accepted scientific methodology

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