

FOREWORD

INTRODUCTION

ISOBUTANOL
CAS N°: 78-83-1

SIDS Initial Assessment Report

For

SIAM 19

Berlin, Germany, 19-22 October 2004

- 1. Chemical Name:** Isobutanol
- 2. CAS Number:** 78-83-1
- 3. Sponsor Country:** United States of America
National SIDS Contact Point in Sponsor Country:
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- 4. Shared Partnership with:** American Chemistry Council, Oxo Process Panel
- 5. Roles/Responsibilities of the Partners:**
 - ∞ Name of industry sponsor /consortium: American Chemistry Council
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 - ∞ 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.

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- 7. Review Process Prior to the SIAM:** Members of the Oxo Process Panel conducted a comprehensive literature search. 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 18.
- 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:** September 2004
- 11. Comments:** Data from the structural analogue n-butanol are used to address the acute aquatic plant endpoint for isobutanol.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	78-83-1
Chemical Name	Isobutanol
Structural Formula	(CH ₃) ₂ -CH-CH ₂ OH

SUMMARY CONCLUSIONS OF THE SIAR**Category/Analogue Rationale**

The acute aquatic plant (green algae) toxicity data on isobutanol (IBOH) was supported/addressed-using data from the structural analog, n-butanol (CAS No. 71-36-3).

Human Health

Isobutanol is rapidly absorbed following inhalation and oral exposures. Isobutanol is rapidly metabolised to isobutyraldehyde and isobutyric acid in rodents and humans.

This chemical has low acute toxicity by all routes. The oral LD₅₀ in male rats is >2830 mg/kg bw and in female rats was 3350 mg/kg bw. Dermal LD₅₀ in male rabbits was >2000 mg/kg bw and 2460 mg/kg bw in female rabbits. Inhalation LC₅₀ values for vapor exposures were >6,000 ppm (18,120 mg/m³) in male and female rats. Isobutanol is a slight to moderate skin irritant and a severe eye irritant.

Repeated exposures to moderate to high concentrations of isobutanol are well tolerated in rats. In a 90-day inhalation study, rats were exposed to isobutanol at 0, 250, 1,000, or 2,500 ppm (760, 3,030 or 7,580 mg/m³). A reduced response to an external stimulus was noted in the exposed animals only during the exposure period. Repeated exposures did not exacerbate these transient effects. There was no evidence of neurotoxicity based on functional observational battery (FOB), quantitative motor activity, neuropathy and scheduled-controlled operant behavior endpoints. The NOAEL was 1,000 ppm (3,030 mg/m³) based on increases in erythrocyte count, hemoglobin, and hematocrit measures in the female rats. Based on the definitive measures of neurotoxicity (FOB, motor activity, histopathology), the NOAEL for neurotoxicity was 2,500 ppm (7,580 mg/m³). A 13-week oral gavage study was conducted with isobutanol with dose levels of 0, 100, 316, and 1,000 mg/kg bw/day. Hypoactivity, ataxia and salivation were noted in the 1,000 mg/kg bw/day dose groups. Hypoactivity and ataxia were resolved by the 4th week of the study. In addition, slight decreases in body weight gain and feed consumption were noted in the first two weeks of the 13-week study in the 1,000 mg/kg bw/day dose group. The NOAEL was 316 mg/kg bw/day.

Several *in vitro* mutagenicity studies indicate that isobutanol 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 (7,580 mg/m³)) did not cause any parental systemic, reproductive, or neonatal toxicity when administered for two generations via whole-body exposure. The NOEL for reproductive and neonatal toxicity was 2,500 ppm (7,580 mg/m³). No adverse developmental effects were noted in rats or rabbits exposed by inhalation to 10,000 mg/m³ isobutanol during gestation. The NOAEL for developmental toxicity was 10,000 mg/m³.

Environment

The available physicochemical data are adequate to describe the properties of isobutanol. Isobutanol has a vapor pressure of 13.9 hPa (10.43 mmHg) at 25°C, a water solubility of 85 g/l at 25°C and a log K_{ow} of 0.79. The melting and boiling points for isobutanol are approximately -108° and 108° C, respectively. The photochemical removal of isobutanol as mediated by hydroxyl radicals occurs with a calculated half-life of 1.55 days. Isobutanol is readily biodegradable under aerobic conditions. Isobutanol volatilises moderately from moving rivers, but less so from quiescent lakes and other surface water bodies (calculated volatilization half-lives of 43 hours from a river and 23

days from a lake). Isobutanol 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 isobutanol released to the environment will partition into water (51.6%) and soil (43.5%), with a smaller amount in air (4.85%).

Acute fish and aquatic invertebrate toxicity data are available for isobutanol. Data from the structure analog n-butanol have been used to support/address the acute aquatic plant endpoint. A flow-through test with fathead minnows (*Pimephales promelas*) reported a 96-hour LC₅₀ of 1430 mg/L. Static tests were conducted using three water column-dwelling invertebrate species (*Daphnia magna*, *D. pulex*, *Ceriodaphnia reticulata*) according to ASTM procedures. Forty-eight hour EC₅₀ values of 1300 (96% CI 1200-1400), 1100 (950-1200), and 1200 (1100-1300) mg/L were reported for the three species, respectively. Since no reliable data are available for describing the toxicity of isobutanol to algae, the results of a test on the structurally analogous substance, n-butanol, are presented. The test with *Selenastrum capricornutum* determined a 96-hour EC₅₀ of 225 mg/l.

Exposure

Isobutanol is manufactured at 16 plant sites in the United States and about 35-40 companies or sites worldwide. Production in the United States was reported to be in the range of 100 – 500 million pounds (45-227 thousand metric tons) in 1998. Worldwide production capacity outside the U.S. is about 402 thousand metric tons. The largest uses of IBOH are as follows: production of isobutyl acetate and other chemicals; use as a direct solvent and as an intermediate in the production of lubricant additives. Use as a direct solvent in coatings, lacquers, primers, and adhesives offers the most potential source of human exposure, since some of these applications are open processes, and isobutanol solvent may be released to ambient air through evaporation as the coating or lacquer dries. Consumers may use some of these products. Human exposure to isobutanol may occur in the work place during manufacture, formulation into products or in various industrial applications, such as working with coatings containing isobutanol as solvent. Such exposures can occur through inhalation and dermal contact. Workplace exposure limits have been established for isobutyl alcohol in most OECD countries. Consumers are exposed when working with consumer products, such as coatings, that contain isobutanol, and through ingestion of foods and beverages that contain naturally occurring isobutanol. Consumers may also be exposed to environmental concentrations of isobutanol in the air or water. Almost all human beings are exposed daily to low concentrations of isobutanol from natural sources, such as in foods and from fermentation of carbohydrates. Exposures to artificial sources also occur, primarily in the vicinities of plants that manufacture, process or use isobutanol in many applications.

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

Human Health: Isobutanol possesses properties indicating a hazard for human health (dermal and eye irritation). These hazards do not warrant further work as they are related to reversible, transient effects that may become evident only at high exposure levels. They should nevertheless be noted by chemical safety professionals and users.

Environment: Isobutanol is currently of low priority for further work due to its low hazard profile.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number:	78-83-1
IUPAC Name:	2-methyl-propan-1-ol
Molecular Formula:	C ₄ H ₁₀ O
Structural Formula:	(CH ₃) ₂ -CH-CH ₂ OH
Molecular Weight:	74.12 g/mol
Synonyms:	isobutyl alcohol IBA, IBOH fermentation butyl alcohol 1-hydroxymethylpropane isobutanol isopropylcarbinol 2-methylpropanol 2-methyl-1-propanol 2-methylpropan-1-ol 2-methylpropyl alcohol

1.2 Purity/Impurities/Additives

No impurities or additives, the purity of isobutanol is greater than 99%.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	Liquid	
Melting point	-108°C	Budavari, S., 1996
Boiling point	108°C	Budavari, S., 1996
Relative density	0.806 at 15°C	Budavari, S., 1996
Vapour pressure	13.9 hPa at 25°C	Daubert, T.E. and R.P. Danner, 1985
Water solubility	85.0 g/l at 25°C	Valvani, S.C, S.H. Yalkowsky, T.J. Rosemand, 1981.
Partition coefficient n-octanol/water (log value)	0.79	BASF AG, 1988
Henry's law constant	1.19x10 ⁻⁵ atm-m ³ /mol	Lyman, W.J., 1982
Flash Point	28° C	NFPA, 2002

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

Isobutanol is a liquid at standard temperature and pressure, with a boiling point of approximately 107° C and a melting point of approximately -108° C (IUCLID, 2003). It is less dense than water

with a specific gravity of 0.806 g/cm³ @ 15° C (Budavari, S., 1996). The solubility limit in water is approximately 85 g/L @ 15° C (Valvani, S.C., 1981). This value indicates isobutanol is very soluble in water.

The vapour pressure of isobutanol is 10.43 mm Hg at 25°C (13.9 hPa) (Daubert and Danner, 1985). Given its solubility limits and its molecular weight of 74.12 g/mole, a Henry's law constant (@ 25° C) was calculated to be approximately 1.19×10^{-5} atm·m³/mole (IUCLID, 2003). In general, chemicals with a Henry's law constant less than 2.0×10^{-5} atm·m³/mole, and a molecular weight less than 200 g/mole tend to partition into water (i.e., are highly water soluble) (Lyman et al., 1982). By this measure, isobutanol is not considered to be a volatile chemical. Isobutanol does, however, meet the definition of a volatile organic compound (VOC).

Isobutanol is flammable with a flash point value of 28°C (82° F). Its lower flammable limit is 1.7% and its upper flammable limit is 10.6%, and has an autoignition temperature of 415° C (780° F) (NFPA, 2002).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Manufacture

Based on the 1998 U.S. EPA Inventory Update Report (IUR), 16 manufacturing facilities produced between 100 million and 500 million pounds (45.4 – 227.3 thousand metric tons) of isobutanol in the United States. According to Bizzari, et al. (2002), the number of producers of isobutanol cited by region or country are: four in Western Europe, 3 in Eastern Europe, 3 in Russia, 1 in Iran, 3 in Japan, 2 in China, 1 in Indonesia, 2 in Korea, and 1 in Brazil. Additional countries import isobutanol for their needs.

Manufacturing capacities expressed in metric tons in these regions or countries in 2002 are estimated to be:

Western Europe	160,000
Eastern Europe	69,000 (including some n-butyl alcohol)
Russia	48,000
Iran	6,000
Japan	43,000
China	14,000
India	8,000 (including some n-butyl alcohol)
Indonesia	10,000
Korea	25,000
Brazil	19,000

Commercial isobutanol is manufactured almost exclusively by the hydrogenation of isobutyraldehyde (Kirk-Othmer, 1991-present), using an enclosed continuous reactor. The material is purified by continuous distillation in an enclosed column. Isobutanol is transported from reactor to distillation column to bulk in-plant storage tanks through pipes. A large portion of isobutanol is

converted at the same plant site to other chemicals, and the remainder is sold. Most isobutanol is shipped in bulk quantities via tank railcar or tank truck. Smaller amounts are transported in closed head steel drums.

Use

In the United States the following breakdown of use percentages is given as follows:

<u>Application</u>	<u>Amount</u>
lube oil additives (in which isobutyl alcohol is an intermediate to produce the lube oil additive ZDDP)	19 thousand metric tons;
conversion to isobutyl acetate - direct solvent -	10 thousand metric tons;
conversion to amino resins	9 thousand metric tons;
conversion to isobutylamines	7 thousand metric tons;
conversion to acrylate and methacrylate esters	1 thousand metric tons;
other uses	1 thousand metric tons

This accounts for 47 thousand metric tons produced in the U.S.

Source: (Bizzari, 2002).

The largest market for isobutanol is to produce zinc dialkyldithiophosphates (ZDDP), which are antiwear and corrosion inhibitor additives for lube oils, greases and hydraulic fluids. The second largest market is conversion to isobutyl acetate. The third largest market is in direct use as a solvent, particularly for surface coatings and adhesives. A major use is as a latent solvent in surface coatings, but it is also used as a processing solvent in the manufacture of pharmaceuticals, pesticides and flavor and fragrances (Bizzari, 2002). Other uses are as a reactive diluent in the alkylation of amino resins, as an industrial intermediate for chemical conversion to isobutylamines, acrylate and methacrylate esters, plasticizers, diisobutyl phthalate, textile chemicals and foundry resin binders. Additionally, isobutanol is used in some foods as a fruit flavoring (Staples, 1998, 1993; Ashford, 1994; and Budavari, S. (ed.). The Merck Index, 1989). Uses of isobutanol in Europe, Japan and other global regions are similar to those in the U.S., however, the percentages for each use vary from region to region (Bizzari, 2002)(Kirk Othmer, 1991-Present)(Furia, TE et al, 1980)(Budavari, S., The Merck Index, 1996). Isobutanol is reported as being used in consumer products (see Section 2.3.2 for further information).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Isobutanol may be present in the environment through releases from waste streams during manufacturing and processing, through use as a direct solvent, and through natural occurrence. Isobutanol is a naturally occurring substance associated with fermentation of carbohydrates, fruits, animal wastes, microbes, and as a plant volatile. It is found in many essential oils, foods and

beverages (Hazardous Substances Data Bank, 2003). The primary route of environmental release in terms of industrial quantities is through evaporation when used as a direct solvent. Use of consumer products containing isobutanol, such as paint and varnish removers can also give rise to consumer and environmental exposure.

Isobutanol is not subject to environmental release reporting under the Toxic Release Inventory requirements. In the Hazardous Substances Data Bank (HSDB), a citation is mentioned that isobutanol has been identified at levels ranging between 142 and 652 ppm in Hyashida River water, which contained effluents from the leather industry (U.S. EPA, 1986).

2.2.2 Photodegradation

The photochemical removal of isobutanol from the troposphere occurs by reaction with hydroxyl radicals. This reaction is the rate-limiting step governing the overall residence time of isobutanol 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 isobutanol. Using a global average tropospheric hydroxyl radical concentration of 1.5×10^6 molecules/cm³, a second order photo-oxidation rate constants of 6.8816×10^{-12} cm³/molecule-sec, and a 12-h daylight period, the total tropospheric half life of isobutanol is expected to be about 1.554 days (37.3 hours) (EPIWIN v.3.10).

2.2.3 Stability in Water

Isobutanol is not expected to hydrolyze in water due to the absence of hydrolysable groups.

2.2.4 Transport between Environmental Compartments

The vapor pressure of isobutanol is 13.9 hPa at 25°C and the water solubility is 85,000 mg/L at 25°C. A Henry's law constant was calculated to be 1.19×10^{-5} atm-m³/mol, using a molecular mass of 74.12 g/mol and the preferred vapor pressure and water solubility. Using W. J. Lyman's Handbook of Chemical Property Estimation Methods as a basis of classification, chemicals with a Henry's Law constant $< 1.0 \times 10^{-3}$ atm/m³/mole, volatilization from water are expected to be moderate. Isobutanol, therefore, would be expected to volatilize only at a moderate rate.

The potential for isobutanol to volatilize from a model river and lake was calculated via EPIWIN (v.3.10) using a water solubility of 85,000 mg/L, a vapor pressure of 13.9 hPa, and a Henry's law constant of 1.19×10^{-5} atm-m³/mol and default model assumptions. Volatilization half-lives from a model river and lake were 43 hours and 23 days, respectively. Thus, volatilization is a minor transport and removal process of isobutanol from surface waters.

The preferred log K_{ow} value is 0.79 (measured, BASF AG, 1988). This octanol/water partition coefficient suggests that isobutanol would not be expected to partition readily from water to soil, sediment, or biota. Similarly, isobutanol in these media would tend to move to water or groundwater if available. Using EPIWIN (v.3.10) and PCKOCWIN (v.1.66), the soil or sediment K_{oc} for isobutanol was calculated to be 2.05 based on the structural features of the molecule. This soil/sediment partitioning values indicate that isobutanol moves fairly readily through soil to groundwater, with little sorption to soil expected.

Fugacity modeling (Level III) was conducted using EPIWIN (v.3.10). Input parameters included molecular weight 74.12 g/mol, melting point -108°C, boiling point 108°C, water solubility 85,000 mg/L, log Kow 0.79, and Henry's law constant 1.19×10^{-5} atm-m³/mol. Equal releases to air, water and soil were assumed. Media-specific half-lives were selected or calculated by the model. The model used a half-life of 37.3 hours for atmospheric photo-oxidation, while biodegradation half-

lives in water, soil and sediment were 360 h, 360 h, and 1440 h, respectively. Biodegradation half-lives were selected by the model based on the biodegradation submodels within EPIWIN (v.3.10). All other parameters used were the model default values. The results support the above conclusions regarding the movement of isobutanol in the environment with 4.85% distributing to air, 51.6% to water, 43.4% to soil and 0.091% to sediment.

2.2.5 Biodegradation

The biodegradation of isobutanol has been reported in several valid tests that were based on specific US or OECD guidelines. The tests mostly involved measuring either the consumption of oxygen (biochemical oxygen demand, BOD) or reduction in dissolved organic carbon (DOC) in vessels containing test substance, non-adapted inoculum from domestic sewage treatment plants, and test media prepared according to the specific method that was used. In a 20-day BOD test, (Price et al., 1974) reported 64% biodegradation by day 5 (as compared to theoretical oxygen demand, thOD of isobutanol is 2.59 mg O₂/mg TS), 73% at day 10, 76% at day 15 and 72% at day 20. Waggy et al. (1994) conducted an OECD 301D Closed Bottle test and reported 14% at day 5, 74% at day 15 and 74% at day 28. Dias and Alexander (1971) conducted a 30-day BOD test at 30°C and reported 42% biooxidation by day 2, 61% by day 5, 75% by day 10 and 55% by day 30. Values were corrected for oxygen consumption in bottles with no test substance present, which accounted for the lower rate on day 30. Huels AG (1978) conducted an OECD 301D Closed Bottle test and reported 55% on day 5, 73% on day 15, and 75% on day 30. These data indicate that isobutanol is readily biodegradable.

The potential for biodegradation in a simulated wastewater treatment plant was examined using the OECD method 303A Coupled Units Test (Huels AG, 1983). In this test, synthetic wastewater with nutrients and TS flow into a 3 L vessel into which air is bubbled simulating aerobic digestion. The temperature is controlled at 21±2°C. Digested wastewater flows into a second vessel in which sludge is allowed to settle and wastewater flows out to a collection vessel. Biodegradation is indicated as DOC reduction and is measured as the difference between initial and final DOC concentrations during the three-hour retention time. Over the course of 35 days, the DOC reduction averaged 97±2.3% (n=24). These data suggest that isobutanol is easily degraded in wastewater treatment plants.

2.2.6 Bioaccumulation

The bioaccumulation potential of isobutanol is low. A measured log K_{ow} value of 0.79 has been reported (BASF AG, 1988). This low octanol:water partitioning coefficient value suggests that isobutanol would not be expected to accumulate in biological tissue or biomagnify in food chains. An estimated bioconcentration factor of 3.2 was calculated using the log K_{ow} value of 0.79, which further suggests a low bioaccumulation potential (EPIWIN v.3.10).

2.3 Human Exposure

Human exposure to isobutanol may occur in work environments, via ingestion of certain foods, or by use of isobutanol-containing products.

2.3.1 Occupational Exposure

Workplace exposure during manufacture or use of isobutanol as an industrial intermediate is limited based on these processes being enclosed, and through engineering controls. For the same reasons, low exposure potential is associated with processes in which isobutanol is used to produce

formulated products. NIOSH, in its NOES Survey (1981-1983) statistically estimated that 192,949 workers (including 28,581 females) were potentially exposed to isobutanol in the U.S. While the NOES survey has flaws in methodology and is outdated, the number may give a magnitude estimate. The American Conference of Governmental Industrial Hygienists (ACGIH) has established a Threshold Limit Value (TLV) of 50 ppm (152 mg/m³) for isobutanol. Other exposure guidelines that have been established include the following:

OSHA PEL of 100 ppm (300 mg/m³)

DFG MAK: 300 mg/m³

Most applications of formulated products containing isobutanol also occur in the workplace. These include application of varnishes and lacquers that contain various concentrations of isobutanol, as well as solvent use in the manufacture of various products in the food, pharmaceutical, and agricultural industries. Since more open processing may occur in the application of varnishes, lacquers and other coatings, the exposure potential is greater. In these cases, the use of spray booths, industrial exhaust systems, and the wearing of protective clothing minimize exposures. Since isobutanol is flammable at a concentration range of 1.7% - 10.6%, precautions are taken to limit open vapour concentrations in the workplace.

2.3.2 Consumer Exposure

Isobutanol is a naturally occurring substance associated with fermentation of carbohydrates, fruits, animal wastes, microbes, and as a plant volatile. Artificial production of isobutanol is used to synthesize esters and polymeric resins. Isobutanol is used as a solvent in lacquers and varnishes. Isobutanol is also used as a diluent in hydraulic fluids. Additionally, isobutanol is used in some foods as a fruit flavouring (Staples, 1998, 1993; Ashford, 1994; and Merck, 1989).

According to the U.S. Environmental Protection Agency SRD, isobutanol is present in some of the following products, which may be used by consumers:

Auto, other transportation, and machinery refinish paints, including primers

Aerosol paint concentrates

Paint and varnish removers

Thinners for dopes, lacquers, and oleoresinous thinners

Insecticides for agriculture, garden and health service use

Writing and stamp pad inks (excluding drawing and printing inks)

Other: art material including clay, water and tempera colors, finger paints, etc.

According to the U.S. National Institutes of Health (NIH) National Household Products Database (accessible online at <http://householdproducts.nlm.nih.gov/products.htm>) isobutanol is present in a number of consumer product formulations, such as the primers and lacquers mentioned above. According to this database, the present concentrations of isobutanol in these products range from 0-4%.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Isobutanol metabolism to isobutyraldehyde and isobutyric acid has been studied in humans, rats, and rabbits.

Studies in Animals

In vitro Studies

The Class I Alcohol Dehydrogenase (ADH) isozymes appear to be the most active for isobutanol metabolism. The alcohol dehydrogenase reaction was studied further in rat and chick embryo liver homogenates (Sinclair, et al., 1990). The clearance of isobutanol in rats was investigated using in situ liver perfusions and in vitro liver homogenates (Hedlund and Kiessling; 1969). Clearance of isobutanol was very rapid in both test systems.

In vivo Studies

Respiratory bioavailability studies conducted with isobutanol have correlated airborne isobutanol levels with internal blood levels of isobutanol and isobutyric acid (Poet, 2003). Inhalation of 2,000 ppm (6,060 mg/m³) isobutanol in a closed chamber resulted in isobutanol levels up to 278 µM and isobutyric acid levels up to 93 µM. Blood levels of isobutanol decreased to 155 µM by ninety minutes and isobutyric acid levels were not detectable. The clearance of isobutanol in rats was investigated using intraperitoneal injections (Hedlund and Kiessling; 1969). Clearance of isobutanol was very rapid. Oral administration of isobutanol to rabbits was reported by Saito (1975), with blood and urinary analysis for isobutanol and metabolites. The metabolism proceeded as expected although the analytical procedures employed detected a urinary metabolite that co-eluted with isovaleric acid but was not fully characterized.

Studies in Humans

In vitro Studies

Metabolism of isobutanol to isobutyraldehyde and isobutyric acid is via the alcohol and aldehyde dehydrogenase enzymes (as was demonstrated in vitro by Ehrig, et al., (1988)). The Class I ADH isozymes appears to be the most active for isobutanol metabolism. The kinetic constants for the alcohol dehydrogenase reaction was determined to have a Km of 0.04- 0.11 µM and a Vmax of 0.68 – 0.86 µmol min⁻¹g wet wt.⁻¹ in human liver homogenates (Sinclair, et al., 1990).

In vivo Studies

Studies in humans (Rudell, et al., 1983) demonstrated the metabolism of isobutanol to isobutyraldehyde and isobutyric acid. Isobutanol is rapidly absorbed following oral administration to humans (Bilzer, et al., 1990).

Conclusion

Isobutanol is rapidly absorbed following oral administration and inhalation exposures. Isobutanol is metabolised to isobutyraldehyde and isobutyric acid in rats and humans, primarily by alcohol and aldehyde dehydrogenases.

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 by OECD Test Guidelines. These values agree with the other acute toxicity data generated prior to promulgation of the test Guidelines (included in the IUCLID).

Studies in Animals

Inhalation

Male and female rats exposed to atmospheric vapor levels of 0, 1500, 3000, or 6000 ppm (0, 4,550, 9,090, 18,120 mg/m³) for six hours were evaluated in a neurobehavioral battery (motor activity determination and a functional observational battery) within two hours post-exposure (Li et al, 1994). Hypoactivity and diminished response to a startle reflex (during the inhalation exposure) was observed during exposure for the 3000 and 6000 ppm (9,090 and 18,120 mg/m³) exposures. Decreases in motor activity were noted post-exposure in the 6000 ppm (18,120 mg/m³) groups but not the 3000 or 1500 ppm (9,090 or 4,550 mg/m³) 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.

Dermal

The dermal LD₅₀ values (24 hour occluded application) for isobutanol in male rabbits was >2000 mg/kg bw and 2460 mg/kg bw in female rabbits (Union Carbide, 1993). Signs of toxicity included sluggishness, prostration, labored breathing and red eyes, and erythema and necrosis at the application site. Skin lesions were still apparent after 14 days.

Oral

The acute oral LD₅₀ value in male rats was >2830 mg/kg bw and in female rats was 3350 mg/kg bw (Christopher, SM. Union Carbide, 1993). Clinical signs associated with oral doses included sluggishness, unsteady gait, lacrimation, piloerection, slow breathing, and prostration. Traces to large amounts of blood were found in the urine.

Studies in Humans

None available.

Conclusion

This chemical has low acute toxicity by all routes. The oral LD₅₀ in male rats is >2830 mg/kg bw and in female rats was 3350 mg/kg bw. Dermal LD₅₀ in male rabbits was >2000 mg/kg bw and 2460 mg/kg bw in female rabbits. Inhalation LC₅₀ values for vapor exposures were >6,000 ppm (18,120 mg/m³) in male and female rats.

3.1.3 Irritation

Skin Irritation

Christopher (1993) reports data from a OECD Guideline 404 acute dermal irritation/corrosion study in rabbits. A 4-hour occluded exposure to 0.5 ml isobutanol produced minor to moderate erythema and edema on 6 of 6 rabbits) within 1 day. Superficial necrosis was noted in 2 of the 6 rabbits. At 7 days, fissuring and desquamation were noted in 1 of 6 and 4 of 6 animals, respectively. By 14 days, alopecia was observed on 2 of 6 rabbits and minor erythema and edema on 1 of 6 rabbits. Isobutanol was considered to be a minor to moderate skin irritant in this study.

Eye Irritation

Christopher (1993) reports data from a OECD Guideline 405 acute eye irritation/corrosion study in rabbits. Installation of 0.1 ml of isobutanol into the conjunctival sac of 2 rabbits caused minor to moderate corneal injury, (including vascularization), iritis, severe conjunctival irritation (including hemorrhages of the nictitating membrane, severe swelling and a pus-like discharge), and alopecia of the periocular area. Minor conjunctival redness was apparent at 21 days. Isobutanol was considered a severe eye irritant in this assay.

Respiratory Tract Irritation

No data available

Conclusion

Isobutanol is a slight to moderate skin irritant and a severe eye irritant.

3.1.4 Sensitisation

No data available.

3.1.5 Repeated Dose Toxicity

Two definitive isobutanol studies were conducted that are considered to be key studies for this endpoint.

Inhalation

One was a 13-week inhalation studies with Sprague-Dawley rats exposed to 0, 250, 1,000 or 2,500 ppm (0, 760, 3,030, or 7,580 mg/m³) (Branch, et al., 1996). This study included expanded neurotoxicity endpoints (functional observational battery, motor activity, scheduled-control operant behavior, and neuropathology endpoints) as well as the standard parameters for subchronic studies. Intensive investigations of testicular parameters (homogenization-resistant spermatid head counts) were collected at necropsy. The highest exposure concentration (2500 ppm; 7,580 mg/m³) did not have any adverse effects demonstrating a persistent or progressive effect of isobutanol on the central or peripheral nervous system. A slight reduction in responsiveness to external stimuli was noted during exposure. Slight increases (9%) in red blood cell parameters (count, hematocrit, hemoglobin) were noted in female rats exposed to 2500 ppm (7,580 mg/m³) but the slight nature of these findings made them of questionable biological significance. There were no changes in any other parameters. The NOAEL for neurotoxicity was 2,500 ppm (7,580 mg/m³). The NOAEL for repeated-dose toxicity was 1,000 ppm (3,030 mg/m³).

Oral

An oral gavage subchronic study has also been reported for isobutanol (Toxicology Research Laboratories, 1987). Groups of thirty male and female rats received isobutanol via oral intubation with dose levels of 0, 100, 316, or 1000 mg/kg bw/day for 90 days. Clinical signs related to treatment with 1000 mg/kg dose level included hypoactivity, ataxia, and salivation. Clinical signs of hypoactivity and ataxia were resolved by the 4th week of the study. Slight decreases in feed consumption and body weight gains were noted in the first two weeks and were restricted to the 1000 mg/kg/day group. There were no changes in organ weights or gross or histopathology at any exposure level. The NOAEL was 316 mg/kg bw/day.

Conclusion

Exposure to high inhalation or oral doses of isobutanol can cause transient, acute decreases in central nervous system function (reduced responsiveness to external stimuli, ataxia, hypoactivity). These effects are not exacerbated by repeated exposures. Repeated exposures to high inhalation levels can cause increases in red blood cell parameters and repeated high oral gavage doses can affect feed consumption and rate of body weight gain. The NOAEL for these endpoints is 1,000 ppm (3,030 mg/m³) (inhalation) or 316 mg/kg bw/day (oral gavage).

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

Isobutanol was not mutagenic in a standard Ames assay when tested at concentrations up to 10,000 µg/plate with and without metabolic activation (Zeiger, et al., 1988, Hazleton Washington, 1992); or a *Escherichia coli* WP2 uvrA gene mutation assay using up to 5,000 µg/plate with and without metabolic activation (Shimizu, et al., 1985). Isobutanol was also negative for genotoxicity in a mouse lymphoma assay using L5178Y cells at levels up to 10 mg/ml (without activation) and 5 mg/ml (with metabolic activation) (Litton Bionetics, 1978). A more recent set of experiments using a Comet assay, a micronucleus assay, and a HPRT-gene mutation assay (all in V79 Chinese hamster fibroblasts) also found isobutanol to be negative for causing genotoxicity at dose levels up to 270 mM (Kreja, and Seidel, 2002).

Experiments using a Comet assay with human lung carcinoma epithelial A549 cells and human peripheral blood cells, also found isobutanol to be negative for causing genotoxicity (Kreja, and Seidel, 2002).

In vivo Studies

The most robust test for genetic toxicity was the oral *in vivo* mouse micronucleus test conducted with isobutanol by the BASF Corporation (Engelhardt and Hoffman, 2000). Isobutanol was administered once orally to male and female NMRI mice at doses up to 2,000 mg/kg body weight. 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

Isobutanol was not genotoxic in *in vitro* experiments using human, rodent, and bacterial cells or *in vivo* experiments in mice.

3.1.7 Carcinogenicity

No reliable data are available.

3.1.8 Toxicity for Reproduction

Effects on Fertility

A two-generation reproductive toxicity study has been conducted by inhalation with isobutanol (WIL Res. Labs, 2003). Groups of male and female rats were exposed by inhalation (6 hours/day, seven days/week) to 0, 500, 1000, or 2500-ppm (1,520, 3,030, or 7,580 mg/m³) isobutanol for two

generations. Daily treatments were continuous with the exception of the period between gestation day 21 thru postnatal day 4 (removal of the dams from the pups during this period typically causes pup mortality). Exposure to 2500-ppm (7,580 mg/m³) isobutanol did not cause any parental systemic, reproductive, or neonatal toxicity when administered for two generations via whole-body exposure. The NOEL was 2500 ppm (7,580 mg/m³).

Developmental Toxicity

In two definitive developmental toxicity studies (BASF a&b, 1990) groups of pregnant female rats (25/group) or rabbits (15/group) were exposed via inhalation to 0, 500, 2,500 or 10,000 mg/m³ (0, 151, 758, or 3030 ppm, respectively) 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 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 was 10,000 mg/m³.

Conclusion

Isobutanol did not cause any reproductive or developmental toxicity in guideline teratology and two-generation reproductive toxicity tests.

3.2 Initial Assessment for Human Health

Isobutanol was only slightly toxic to experimental animals following acute oral, dermal, or inhalation exposure. Isobutanol is a slight to moderate skin irritant and a severe eye irritant. Repeated dose toxicity studies by the inhalation and oral routes of exposure had NOEL's of 1000 ppm (3,030 mg/m³) and 316 mg/kg bw/day, respectively. The NOAEL for neurotoxicity in a 90-day study was 2500 ppm (7,580 mg/m³). A two-generation reproductive toxicity study indicates that isobutanol is not a reproductive toxicant. Isobutanol produced no fetotoxicity or developmental anomalies at or near maternally toxic doses. Several in vitro tests and an in vivo micronucleus test indicate that isobutanol is not genotoxic.

4 HAZARDS TO THE ENVIRONMENT

Analog Justification

A number of aquatic toxicity data are available for isobutanol. However, as explained below, data from the acute aquatic plant studies for isobutanol do not meet current OECD requirements. Therefore, based on structural similarities and carbon chain length, data for n-butanol (CAS# 71-36-6) are considered suitable to serve as a structural analog for isobutanol. For comparison purposes only, data from n-butanol have been presented for the remaining acute fish and aquatic invertebrate endpoints.

4.1 Aquatic Effects

Valid acute aquatic toxicity data are available for isobutanol with fish and aquatic invertebrates.

Fish

A flow through test using the fathead minnow *Pimephales promelas* was conducted using US EPA procedures (Brooke et al., 1984). A 96-hour LC₅₀ for lethality and lethargy of 1430 (95% CI 1370-1490) mg/L was reported.

Aquatic Invertebrates

As part of a cooperative research program with the US EPA, Elnabaraway et al. (1986) conducted static tests using three water column invertebrate species (*Daphnia magna*, *D. pulex*, *Ceriodaphnia reticulata*). Tests were conducted according to ASTM procedures (ASTM 1984a,b). Forty-eight hour EC₅₀ values of 1300 (96% CI 1200-1400), 1100 (950-1200), and 1200 (1100-1300) mg/L were reported for *D. magna*, *D. pulex*, and *C. reticulata*, respectively.

Algae

[These studies have reliability code of 4.]

Due to non-OECD guideline study duration and the lack of experimental details available for the studies by Bringmann and Kuhn (1978) and Kuhn and Petard (1990) that used green algae with isobutanol, data from an appropriate structural analog is presented to assist in addressing the ecotoxicity of isobutanol. Analog data are presented in Table 2. Using n-butanol, a static study with the green alga *Selenastrum capricornutum* was conducted using OECD method 201, reporting a 96-hour EC₅₀ of 225 (95% CI 204-246) mg/L (Wong et al., 1998).

Since isobutanol is only slightly volatile, tests conducted under static conditions are unlikely to be affected by volatilization. As previously indicated in the SIAR, the calculated half-life of isobutanol from a quiescent lake is 23 days. Volatilization is a function of the exchange of gases across the water surface film. This exchange is a direct function of the actual wind velocity. Studies performed under static test conditions use test vessels that are kept in constant temperature incubators with essentially no wind or air flow. As a result, the volatilization of the test substance is minimized. By way of comparison, the calculated half-life from a quiescent lake for n-butanol is 436 days. Therefore, volatilization should not affect the results from static or static renewal tests with isobutanol or its analog, n-butanol. Some of the measured concentrations in the fish study were <80% of nominal, which was probably due to a combination of biodegradation and the slight amount of expected volatilization.

In addition, to further support the measured data from static tests, estimated values using ECOSAR (v. 0.99g) are provided in Table 2. Taking the available measured and estimated data together; the acute toxicity of isobutanol to fish, aquatic invertebrates and algae can be reliably determined. The measured test results for isobutanol and its analog indicate that acute toxicity to aquatic life would occur at concentrations ranging between 225 to 1430 mg/L.

Table 2. Ecotoxicity data for isobutanol and analog (effect concentrations all mg/L)

	Isobutanol	n-Butanol
Properties	C ₄ H ₁₀ O CAS# 78-83-1	C ₄ H ₁₀ O CAS# 71-36-3
Endpoint		
Fish	<i>Pimephales promelas</i> 96-h LC ₅₀ = 1430 mg/L	<i>Pimephales promelas</i> 96-h LC ₅₀ = 1376* mg/L
Fish - ECOSAR	96-h LC ₅₀ = 754 mg/L	96-h LC ₅₀ = 621* mg/L
Aquatic Invertebrate	<i>Daphnia magna Straus</i> 48-h EC ₅₀ = 1300 mg/L <i>Daphnia Pulex</i> 48-h EC ₅₀ = 1100 mg/L <i>Ceriodaphnia reticulata</i> 48-h EC ₅₀ = 1200 mg/L	<i>Daphnia magna</i> 48-h LC ₅₀ = 1328* mg/L
Daphnid - ECOSAR	48-h EC ₅₀ = 743 mg/L	48-h EC ₅₀ = 615* mg/L
Green Algae	not adequate	96-h EC ₅₀ = 225 mg/L
Green algae - ECOSAR	96-h EC ₅₀ = 433 mg/L	96-h EC ₅₀ = 361 mg/L

*For comparison purposes butanol data has been included in the above table. However, the fish and invertebrate data for n-butanol were not used to fulfil the fish and invertebrate endpoints for isobutanol.

4.2 Terrestrial Effects

No ecotoxicological data for isobutanol were identified for terrestrial wildlife (*i.e.*, birds and mammals) or other terrestrial organisms (*e.g.*, plants, invertebrates, and bacteria).

4.3 Other Environmental Effects

No data.

4.4 Initial Assessment for the Environment

The available physicochemical data are adequate to describe the properties of isobutanol. Isobutanol has a vapor pressure of 13.9 hPa (10.43 mmHg) at 25°C, a water solubility of 85 g/l at 25°C and a log K_{ow} of 0.79. The photochemical removal of isobutanol as mediated by hydroxyl radicals occurs with a calculated half-life of 1.55 days. Isobutanol is readily biodegradable under aerobic conditions. Isobutanol volatilises moderately from moving rivers, but less so from quiescent lakes and other surface water bodies (calculated volatilization half-lives of 43 hours from a river and 23 days from a lake). Isobutanol 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 isobutanol released to the environment will partition into water (51.6%) and soil (43.5%), with a smaller amount in air (4.85%).

Acute Aquatic fish and aquatic invertebrate toxicity data are available for isobutanol. Data from the structure analog n-butanol have been used to support/address the acute aquatic plant endpoint. A flow-through test with fathead minnows (*Pimephales promelas*) reported a 96-hour LC₅₀ of 1430

mg/L. Static tests were conducted using three water column-dwelling invertebrate species (*Daphnia magna*, *D. pulex*, *Ceriodaphnia reticulata*) according to ASTM procedures. Forty-eight hour EC₅₀ values of 1300 (96% CI 1200-1400), 1100 (950-1200), and 1200 (1100-1300) mg/L were reported for the three species, respectively. Since the duration of the green algal study did not meet the OECD guidelines (duration of study and uncertainties in study details, data from an analogous compound, n-butanol (CAS No. 71-36-3) are presented. A 96-hour EC₅₀ of 225 mg/L was reported for the green alga *Selenastrum capricornutum*, toxicity endpoint.

Photochemical Ozone Creation Potential (POCP) is a measure of the relative potential of a chemical to form ozone in the atmosphere. POCP is not measured directly but rather is developed from atmospheric and chemical mechanistic models. As a result, reported POCP values for a single chemical may vary considerably with atmospheric conditions including meteorology, amount of sunlight, and the concentration of nitrogen oxides and other volatile organic compounds already in and being newly emitted to the air. POCP values for isobutanol ranging from around 25 to 60 can be found in the literature. A representative value of 37.5 (relative to ethene) is found in R. G. Derwent, et al., Photochemical Ozone Creation Potentials for Organic Compounds in Northwest Europe Calculated with a Master Chemical Mechanism, *Atmospheric Environment*, Vol. 32, No. 19, 1998.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

The chemical possesses properties indicating a hazard for human health (dermal and eye irritation). Although these hazards do not warrant further work (as they are related to reversible, transient effects that may become evident only at very high exposure levels), they should nevertheless be noted by chemical safety professionals and users.

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**ROBUST SUMMARIES and
SIDS DOSSIER for:
ISOBUTANOL**

.....

CAS No. 78-83-1

Sponsor Country: U.S.A.

DATE: Updated September 2004

1.0 GENERAL INFORMATION**1.01 SUBSTANCE INFORMATION**

A.	CAS-Number	78-83-1
B.	Name (<i>IUPAC name</i>)	2-methyl-propan-1-ol
C.	Name (<i>OECD name</i>)	Isobutanol
D.	CAS Descriptor	Not applicable in this case
E.	EINECS-Number	201-148-0
F.	Molecular Formula	C ₄ H ₁₀ O
G.	Structural Formula	(CH ₃) ₂ -CH-CH ₂ OH
H.	Substance Group	N/A
I.	Substance Remark	None
J.	Molecular Weight	74.12 g/mol

1.02 OECD INFORMATION

A.	Sponsor Country:	U.S.A.
B.	Lead Organisation: Name of Lead Organisation: Contact person: Address:	American Chemistry Council Doug Anderson 1300 Wilson Blvd. Arlington, VA 22209 U.S.A. Tel: 703-741-5000 Fax: 703-741-6000

1.1 GENERAL SUBSTANCE INFORMATION

A.	Type of Substance	element []; inorganic []; natural substance []; organic [X]; organometallic []; petroleum product []
B.	Physical State (<i>at 20°C and 1.013 hPa</i>)	gaseous []; liquid [X]; solid []

- C. **Purity** (*indicate the percentage by weight/weight*)
>99%

1.2 SYNONYMS

isobutyl alcohol
IBA
Fermentation butyl alcohol
1-hydroxymethylpropane

isopropylcarbinol
2-methylpropanol
2-methyl-1-propanol
2-methylpropan-1-ol
2-methylpropyl alcohol

1.3 IMPURITIES

No data available

1.4 ADDITIVES

None

1.5 QUANTITY

Production Volume

In U.S.: 100,000,000-500,000,000 pounds (45.4-227.3 thousand metric tons)

Year: 2002

Source: U.S. EPA Inventory Update Report (IUR)

Production Volume

Outside U.S.: Ca. 402,000 metric tons

Year: 2002

Source: Bizziari, S.N., R. Gubler, and A. Kishi. CEH Marketing Research Report for Plasticizer Alcohols. May 2002.

1.6 LABELLING AND CLASSIFICATION

No data available

1.7 USE PATTERN

A. General

Type of Use: Industrial
 Category: Basic industry, basic chemicals
 Remark: Largest use is to make lubrication oil additives
 Source: Bizziari, S.N., R. Gubler, and A. Kishi. CEH Marketing Research Report for Plasticizer Alcohols. May 2002.

Type of Use: Industrial
 Category: Chemical Industry: Used in synthesis
 Remark: Chemical intermediate to manufacture isobutyl acetate, isobutylamines, acrylate and methacrylate esters, plasticizers, diisobutyl phthalate, textile chemicals and foundry resin binders.
 Source: Bizziari, S.N., R. Gubler, and A. Kishi. CEH Marketing Research Report for Plasticizer Alcohols. May 2002.

Type of Use: Industrial
 Category: Basic Industry; Basic Chemicals
 Remark: Direct solvent, particularly for surface coatings and adhesives. A major use is as a latent solvent in surface coatings, but is also used as a processing solvent in the manufacture of pharmaceuticals, pesticides and flavor and fragrances.
 Source: Bizziari, S.N., R. Gubler, and A. Kishi. CEH Marketing Research Report for Plasticizer Alcohols. May 2002.

Type of Use:
 Category:
 Remark: Isobutanol is used in some foods as a food flavorant
 Sources: Staples, 1998, 1993; Ashford, 1994; and Budavari, S. (ed) The Merck Index, 1989.

B. Uses in Consumer Products

Type of Use: Other: Use in Consumer Products
 Category:
 Remark: Isobutyl Alcohol is present in the following products, which may be used by consumers:

Auto, other transportation and machinery refinish paints, including primers
 Aerosol paint concentrates
 Paint and varnish removers
 Thinners for dopes, lacquers, and oleoresinous thinners
 Insecticides for agriculture, garden, and health service use
 Writing and stamp pad inks (excluding drawing and printing inks)
 Other art material including clay, water, and tempera colors, finger paints, etc.

The concentration in these products is reported to range from 0-4%

Source: Environmental Protection Agency. 1986. Health and Environmental Profile for Isobutyl Alcohol. ECAO-CIN-P171. SRD; U.S. National Institutes of Health (NIH) National Household Products Database (accessible online at <http://householdproducts.nlm.nih.gov/products.htm>)

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

Type of Limit:	U.S. ACGIH 8 hour TLV
Value:	50 ppm 152 mg/m ³
Reference:	ACGIH TLVs and BEIs Handbook (1997).
Type of Limit:	U.S. OSHA PEL
Value:	100 ppm (300 mg/m ³)
Reference:	ACGIH, 2002. Guide to Occupational Exposure Values – 2002. American Conference of Governmental industrial Hygienists, Inc. (ACGIH). Cincinnati, OH
Type of Limit:	U.S. NIOSH REL
Value:	50 ppm (152 mg/m ³)
Reference:	ACGIH, 2002. Guide to Occupational Exposure Values – 2002. American Conference of Governmental industrial Hygienists, Inc. (ACGIH). Cincinnati, OH
Type of Limit:	DFG MAK
Value:	100 ppm (310 mg/m ³)
Remark:	DFG Category I: Substance for which local irritant effects determine the MAK value.
Reference:	ACGIH, 2002. Guide to Occupational Exposure Values – 2002. American Conference of Governmental industrial Hygienists, Inc. (ACGIH). Cincinnati, OH

1.9 SOURCES OF EXPOSURE

Remark:	Isobutyl alcohol may be present in the environment through releases from waste streams during manufacturing and processing, through use as a direct solvent, and through natural occurrence. IBA is a naturally occurring substance associated with fermentation of carbohydrates, fruits, animal wastes, microbes, and as a plant volatile. It is found in many essential oils, foods and beverages (Hazardous Substances Data Bank, 2003). The primary route of environmental release in terms of industrial quantities is through evaporation when used as a direct solvent.
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1.10 ADDITIONAL REMARKS

No additional remarks.

2.0 PHYSICAL-CHEMICAL DATA**2.1 MELTING POINT**

- (a) Preferred value
Value: -108° C
Remark:
Reliability: Score=2, valid with restrictions
Reference: Budavari, S. (edi.) 1996. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, NJ.
- (b) Value: -108° C
Reliability: Score = 2, valid with restrictions
Reference: Aldrich Catalogue, 2003-2004. p. 1280
- (c) Value: -108° C
Reliability: Score = 2, valid with restrictions
Reference: Patty's Industrial Hygiene and Toxicology (1982), 3rd Edition. Volume 2C, p. 4578. John Wiley and Sons.

2.2 BOILING POINT

- (a) Preferred result
Value: 108° C
Reliability: Score = 2, valid with restrictions
Reference: Budavari, S. (edi.) 1996 The Merck Index. An Encyclopedia of Division of Merck & Co., Inc. Whitehouse Station, NJ.
- (b) Value: 108° C
Reliability: Score = 2, valid with restricaitions
Reference: Aldrich Catalogue, 2003-2004. p. 1280
- (c) Value: 108° C
Reliability: Score = 2, valid with restrictions
Reference: Patty's Industrial Hygiene and Toxicology (1982), 3rd Edition. Volume 2C, p. 4578. John Wiley and Sons.

2.3 DENSITY

- (a) Preferred result
Value: 0.806 g/cm³
Temperature: 15° C
Remark:
Reliability: Score=2, handbook of data
Reference: Budavari, S. (edi.) 1996. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, NJ
- (b) Preferred value:
Value: 0.8018 g/cm³
Temperature: 20° C
Method: No data available
Year: No data available

GLP: No data available
 Reliability: Score=2, valid with restrictions, no experimental details available
 Reference: CRC Handbook of Chemistry and Physics. 1995-1996. D.R. Lide (ed.).
 76th ed. CRC Press, Inc. Boca Raton, FL.

2.4 VAPOUR PRESSURE

- (a) Preferred value
 Value: 13.9 hPa (10.43 mm Hg)
 Temperature: 25° C
 Reliability: Score = 2, valid with restrictions
 Reference: Daubert, T.E. and R.P. Danner. Data Compilation Tables of Properties of Pure Compounds. 1985. Design Institute for Physical Property Data, American Institute of Chemical Engineers.
- (b) Value: 16.27 hPa (12.2 mmHg)
 Reliability: Score=2, valid with restrictions, handbook of data
 Reference: Patty's Industrial Hygiene and Toxicology (1982), 3rd Edition, Volume 2 C, p. 4578. John Wiley and Sons.
- (c) Value: 15.27 hPa (11.45 mm HG)
 Reliability: Score = 2, valid with restrictions
 Reference: Riddick, Bunger, and Sakano (1986). Organic Solvents Physical Properties and Methods of Purification, 4th Edition, Volume II. P. 201.
- (d) Value: 13.3 hPa (10 mm Hg)
 Temperature: 21.7°C
 Reliability: Score = 2, valid with restrictions, handbook of data
 Reference: Sax and Lewis, Sr. 1989. Dangerous Properties of Industrial Materials. 7th Edition. P. 2020. Van Nostrand Reinhold.
- (e) Value: 14 hPa (10.5 mm Hg)
 Temperature: 25° C
 Reliability: Score = 2, valid with restrictions
 Reference: SRC Physical Properties database on-line.
<http://www.syrres.com/esc/physdemo.htm>

2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

- (a) Preferred value:
 $\log P_{ow}$: 0.79 at 25° C
 Remark: OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
 Reliability: Score=2, valid with restrictions, safety data sheets
 Reference: BASF AG Ludwigshafen
 ECB - Existing Chemicals Ispra (VA)
 Hoechst AG Frankfurt/Main
 Celanese, N.V. Rotterdam
 Celanese GmbH Frankfurt am Main
 BASF AG, Analytisches Labor; unveroeffentlichte Untersuchung (J.Nr.124835/01) vom 26.05.88
- (b) $\log P^{ow}$: 0.76
 Reliability: Score = 2, valid with restrictions

- Reference: Hansch, Leo, and Hoekman (1995). Exploring USAR, Hydrophobic, Electric, and Steric Constance. ACS Professional Reference Book, American Chemical Society, Washington DC.
- (c) log P_{ow}: 0.79
 Reliability: Score = 2, valid with restrictions
 Reference: Handbook of Environmental Data on Organic Chemicals, 4th Edition, Volume II. 2001. p. 1328, John Wiley and Sons.

2.6 WATER SOLUBILITY

- (a) Preferred result
 Value: 85 g/L at 25° C
 Remark: 85,000 mg/L at 25° C
 Reference: Valvani, S.C., S.H. Yalkowsky, T.J. Rosemand. Solubility and Partitioning. IV. Aqueous Solubility and Octanol-Water Partition Coefficients of Liquid Non-electrolytes. J. Pharm. Sci. 70: 502-7.
- (b) Value: 50,000 mg/L
 Remark: 50 g/L
 Reference: Budavari, S. (edi.) 1996. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, NJ
- (c) Value: 100,000 mg/L
 Remark: 100 g/L
 Reference: Riddick, Bunger, and Sakano (1986). Organic Solvents Physical Properties and Methods of Purification, 4th Edition, Volume II. p. 201.
- (d) Value: 100,000 mg/L
 Remark: 100 g/L
 Reference: Ashford's Dictionary of Industrial Chemicals (2001), 2nd Edition, p. 634. Wavelength Publicaitons.
- (e) Value: 95,000 – 100,000 mg/L
 Remark: 95-100 g/L
 Reference: Patty's Industrial Hygiene and Toxicology. 1982. 3rd Edition. Volume 2C, p 4578. John Wiley and Sons.
- (f) Value: 85,000 mg/L
 Remark: 85 g/L
 Reference: Handbook of Environmental Data on Organic Chemicals, 4th Edition, Volume II. 2001. p. 1328, John Wiley and Sons.
- (g) Value: 85,000 at 20 deg. C
 Remark: 85 g/L
 Reference: Hazardous Substance Data Bank (HSDB) Accessible online at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/search>

2.7 FLASH POINT (*liquids*)

- (a) Preferred result
 Value: 28° C

Remark:	82° F – closed cup
Reliability:	Score=2, valid with restrictions, handbook of data
References:	Budavari, S. (edi.) 1996 The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, NJ.
	Riddick, Bunger, and Sakano. 1986 Organic Solvents Physical Properties and Methods of Purification. 4 th Edition, Volume II. p. 201.
	Aldrich Catalogue (2003-2004) p. 1280.
	Documentation of the Threshold Limit Values and Biological Exposure Indices (1991) 6 th edition, Volume II. p. 815. American Conference of Industrial Hygienists, Inc. Cincinnati, Ohio.
(b) Value:	35° C
Remark:	95° F – (TOC)
Reliability:	Score=2, valid with restrictions, handbook of data
Reference:	Riddick, Bunger, and Sakano. 1986 Organic Solvents Physical Properties and Methods of Purification. 4 th Edition, Volume II. p. 201.
(c) Value:	37.8° C
Remark:	100° F (open cup)
Reliability:	
Reference:	Documentation of the Threshold Limit Values and Biological Exposure Indices (1991) 6 th edition, Volume II. p. 815. American Conference of Industrial Hygienists, Inc. Cincinnati, Ohio.

2.8 AUTO FLAMMABILITY (solid/gases)

No data available

2.9 FLAMMABILITY

Flash Point

Value: (closed cup) 28 degrees C, 82 degrees F

Remark: Autoignition temperature: 780 degrees F, 415 degrees C

LFL (lower flammable limit): 1.7% by volume (17,000 ppm) at 51° C.

UFL (upper flammable limit) 10.6% by volume (106,000 ppm) at 94° C

Reference: NFPA, 2002 Fire Protection Guide to Hazardous Materials, 13th Edition.

National Fire Protection Association, Quincy, MA

2.10 EXPLOSIVE PROPERTIES

- | | |
|----------------------|--|
| (a) Explosive Limit: | LFL (lower flammable limit) 1.7% by volume (17,000 ppm) at 51° C
UFL (upper flammable limit) 10.6% by volume (106,000 ppm) at 95° C |
| Reference: | Montgomery, J. Groundwater Chemicals Desk Reference. 1996. 2 nd edition, p. 953, CRC Press. |
| (b) Explosive Limit: | LFL (lower flammable limit) 1.2% by volume (12,000 ppm)
UFL (upper flammable limit) 10.9% by volume (109,000 ppm) at 100° C |
| Reference: | Sax and Lewis, Sr. 1989. Dangerous Properties of Industrial Materials. 7 th edition, p. 2020. Van Nostrand Reinhold. |

2.11 OXIDIZING PROPERTIES

No data available

2.12 ADDITIONAL REMARKS

No data available

2.13 ADDITIONAL DATA

- (a) Type: Henry's Law constant
Test substance: isobutanol
Method: Calculated using water solubility 85,000 mg/L, vapor pressure (10.43 mm Hg), and molecular weight 74.12 g/mol.
Result: 1.19×10^{-5} atm-m³/mol
GLP: Not applicable
Reference: Lyman, W.J., et al. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill NY
- (b) Type: Viscosity Coefficient cP
Value: 3.333
Reliability: Score = 2, valid with restrictions
Reference: Riddick, Bunger, and Sakano. 1996. Organic Solvents Physical Properties and Methods of Purification. 4th edition, Volume II. p. 201
- (c) Type: Refractive Index, n_D at 20 °C
Value: 1.396
Reliability: Score = 2, valid with restrictions
Reference: Aldrich Catalogue (2003-2004) p. 1280
- (d) Type: Surface Tension dyne/cm
Value: 22.98 at 20 °C
Reliability: Score=2, valid with restrictions
Reference: Riddick, Bunger, and Sakano. 1996. Organic Solvents Physical Properties and Methods of Purification. 4th edition, Volume II. p. 201.
- (e) Type: Evaporation Rate (BuOAc = 1)
Value: 0.62
Reliability: Score=2, valid with restrictions
Reference: Riddick, Bunger, and Sakano. 1996. Organic Solvents Physical Properties and Methods of Purification. 4th edition, Volume II. p. 201.
- (f) Type: Dielectric Constant
Value: 17.93
Reliability: Score=2, valid with restrictions
Reference: Riddick, Bunger, and Sakano. 1996. Organic Solvents Physical Properties and Methods of Purification. 4th edition, Volume II. p. 201.
- (g) Type: Vapor Density (air = 1)
Value: 2.55
Reliability: Score=2, valid with restrictions
Reference: Documentation of the Threshold Limit Values and Biological Exposure Indices. 1991. 6th edition, Volume 2, p. 815. American Conference of Industrial Hygienists, Inc. Cincinnati Ohio.

3.0 ENVIRONMENTAL FATE AND PATHWAYS**3.1 STABILITY****3.1.1 PHOTODEGRADATION**

- (a) Preferred value
- | | |
|-----------------|---|
| Type: | Other; see remarks |
| Light Source: | |
| Light spect.: | nm |
| Rel. intensity | based on intensity of sunlight |
| Degradation: | |
| Method: | other (calculated): AOPWIN v1.90 |
| GLP: | no |
| Test substance: | isobutanol |
| Remark: | Vapor phase isobutanol is susceptible to reaction with photochemically produced hydroxyl (OH) radicals. The 2nd order rate constant for reaction with hydroxyl radicals was calculated as $6.88E-12$ cm ³ /(molecule-sec). Based on $1.5E6$ OH molecules/cm ³ and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 37.3 hours. |
| Reliability: | Score=2, valid with restrictions |
| Reference: | AOPWIN. Version 1.90. Atmospheric Oxidation. EPIWIN v.3.10 (Estimation Program Interface for Windows). US. Environmental Protection Agency (2000) |

3.1.2 STABILITY IN WATER

Isobutanol is not expected to hydrolyze in water due to the absence of hydrolysable groups.

3.1.3 STABILITY IN SOIL**3.2 MONITORING DATA (ENVIRONMENT)****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: | isobutanol |
| Method: | Calculated using EPISUITE v3.10 (USEPA, 2001) |
| Result: | Half-life from model river: 43 hours
Half-life from model lake: 23 days |
| Remark: | Based on Henry's law constant of $1.19 E^{-5}$ atm-m ³ /mol, vapor pressure of 10.43 mm Hg, water solubility of 85,000 mg/L, and a molecular weight of 74.12 g/mole, and model defaults (for model river: river 1 m 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). Using W. J. Lyman's Handbook of Chemical Property Estimation Methods as a basis of classification, chemicals with a Henry's Law constant $<1.0 \times 10^{-3}$ atm/m³/mole, volatilization from water are expected to be moderate. Isobutanol, therefore, would be expected to volatilize only at a moderate rate.

GLP: Not applicable
 Reliability: Score=2, valid with restrictions, calculation
 References: EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).

Lyman, W.J., et al. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill NY.

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

(a) Preferred result
 Type: Level III Fugacity-based distribution modeling
 Test substance: Isobutanol
 Method: Level III fugacity based model, EPISUITE 3.10.
 GLP: Not applicable
 Remark: Default values were assumed for environmental compartment descriptions, dimensions, and properties, advective and dispersive properties. Chemical-specific input parameters were: molecular weight (74.12 g/mol), vapor pressure (10.43 mm Hg), log K_{ow} (0.79), melting point -108 deg. C, aqueous solubility 85,000 mg/L, boiling point of 107 deg. C, and a Henry's Law constant of 1.19×10^{-5} atm-m³/mol. Half-lives calculated by the model based on the properties of the test substance were: water and soil half-lives 360 hr, and sediment half-life 1440 hr. The half-life in air was 37.3 hours and was based on a second-order rate constant for atmospheric hydroxy radical-mediated photo-oxidation of 6.88×10^{-12} cm³/molecule-sec, a 12 hour day and a hydroxy radical concentration of 1.5×10^6 molecules/cm³. Physical properties were the preferred values from the SIDS dossier. Emissions were assumed to be equally to air, water and soil. Overall persistence was 268 hours.
 Distribution: Air (4.85%), Water (51.6%), Soil (43.4%), Sediment (<0.1%)
 Reliability: Score=2 is assigned a result using an accepted method of estimation. No measured data available to confirm the calculated value.
 Reference: EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).

3.3.3 OTHER DISTRIBUTION

(a) Type: Soil or sediment partition coefficient (K_{oc})
 Test substance: isobutanol
 Method: Calculated using EPISUITE v.3.10 and PCKOCWIN v.1.66 using structural features of the molecule
 Result: 2.05 L/kg
 GLP: Not applicable
 Reliability: Score=2, valid with restrictions, calculation
 Reference: EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

3.5 BIODEGRADATION

- (a) 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: 72% after 20 days
 Result: readily biodegradable
 Kinetic of test sub.: 5 day = 64%, 10 day = 73%, 15 day = 76%, 20 days = 72%
 Method: BOD (Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edi. American Public Health Association, New York, NY)
- 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 available
 Test substance: isobutanol (2-methyl-1-propanol)
 Remark: Typical unacclimated biodegradation curves for alcohols were provided. The biodegradation curve for isobutanol showed steadily increasing oxidation from test initiation to Day 10, followed by a plateauing through day 20. Isobutanol is readily biodegradable. Measured COD was reported as 2.39 mg/mg; the theoretical oxygen demand was reported as 2.59 mg/mg.
 Reliability: Score=2 valid with restrictions, not all experimental details available
 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: activated sludge effluent, non-adapted
 Concentration: 2 mg/L
 Contact time: 28 days
 Degradation: 74% after 20 days
 Results: readily biodegradable
 Kinetic of test subst: 5 day = 14%, 15 day = 74%, 28 days = 74%
 Method: OECD 301D, Closed Bottle Test, OECD (1989), Paris
 Coarse-filtered mixture of domestic treatment plant effluents and rich soil microorganisms were added to BOD dilution water at a concentration of 0.1 mL per liter. BOD dilution water is fortified with specified minerals and buffered to pH 7.2. Seven BOD bottles were prepared with and without test substance added. One was measured for DO immediately and duplicate bottles measured at days 5, 15, and 28 using a YSI dissolved O₂ meter. Bottles were incubated at 20° C. DO measurements for the test and standard substance (ethylene glycol) were corrected for the blank values.
- Year: 1993
 GLP: no data available
 Test substance: isobutanol (2-methyl-1-propanol)
 Reliability: Score=2, valid with restrictions, not all experimental details available

Reference:	Waggy FT, Conway RA, Hansen JL, Blessing RL. 1994. Comparison of 20-d BOD and OECD Closed-Bottle Biodegradation Tests. <i>Environ Toxicol Chem</i> , 13: 1277-1280.
(c) Type:	aerobic
Inoculum:	domestic sewage effluent, non-adapted
Concentration:	3.08 mg/l related to DOC (Dissolved Organic Carbon) (2 mg C/L)
Contact Time:	30 days
Degradation:	75% after 10 days
Results:	readily biodegradable
Kinetic of test subst:	2 day = 42%, 5 day = 61%, 10 days = 75%, 30 days = 55%
Method:	Oxygen Consumption Test Raw sewage was filtered through cotton and added to BOD dilution water at a concentration of 5 mL per liter. BOD dilution water is fortified with specified minerals and buffered. Bottles were incubated in the dark at 25 deg. C. DO measurements for the test substance and standard substance (glucose) were corrected for the blank values (inoculum-only). Oxygen depletion was further corrected for nitrification. The nitrification occurred due to the presence of nitrogen-containing materials in the sewage sludge seed. Positive control (glucose) results were not separately reported.
Year:	1971
GLP:	no data
Test substance:	isobutanol (2-methyl-1-propanol)
Reliability:	Score=2, valid with restrictions, not all experimental details available
Reference:	Dias, E.F. and M. Alexander. 1971. Effect of Chemical Structure on the Biodegradability of Aliphatic Acids and Alcohols. <i>Applied Microbiology</i> . 22(6):1114-1118.
(d) Type:	aerobic
Inoculum:other:	Sewage sludge from a municipal sewage treatment in Marl, Germany, non-acclimated
Concentration:	2 mg/L
Contact Time:	30 days
Degradation:	75% after 30 days
Results:	readily biodegradable
Kinetic of test Subst:	5 day = 55%, 15 day = 73%, 30 days = 75%
Method:	OECD 301D, Closed Bottle Test Wastewater from a domestic treatment plant (Marl-West, Germany) were added to BOD dilution water at a concentration of 0.5 mL per liter. BOD dilution water is fortified with specified minerals and buffered to pH 7.2. Replicate BOD bottles were prepared with and without test substance added or with Texapon as a positive control. DO was measured with an O2 meter on days 5, 15, and 30. Bottles were incubated at 20 deg. C. DO measurements in the bottles without any TS showed O2 consumption of 0.9 mg/L (below the maximum desired O2 consumption for blanks of 1.5 mg/L). Percent degradation was calculated as percent theoretical oxygen demand (2.59 mg O2/mg TS).
Year:	1978
GLP:	no data
Test substance:	isobutanol (2-methyl-1-propanol)
Reliability:	Score=2 valid with restrictions, not all experimental details available

Reference:	Huels AG, 1978, Abschlussbericht GF-108. Bestimmung der biologischen Abbaubarkeit von Isobutanol im Geschlossenen Flaschentest (OECD-method 301D), Marl, Germany.
(e) Type:	aerobic
Inoculum:	other: Sewage sludge from a municipal sewage treatment in Marl, Germany
Concentration:	12.4 mg/L related to DOC (Dissolved Organic Carbon)
Degradation:	96.98±2.3 % per 3 hour turnover during the course of 35 day(s)
Method other:	OECD Guide-line 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Unit Test"
	<p>In a coupled-unit test, stock solution consisting of nutrient solution plus test substance is pumped into a 3L reactor into which air is also pumped providing air and agitation. The reactor has been seeded with synthetic wastewater and activated sludge from a municipal sewage treatment plant (Marl-West, Germany). The treated water flows into a second vessel that is not agitated. Within the second vessel, the sludge settles and the remaining water drains off into a collection vessel. The flow-through time is 3 hours. Test substance is measured at the stock vessel and the final collection vessel. Twenty-four measurements were made over the course of 35 days. The exact recipe for the synthetic wastewater and nutrient solutions are given in the report and OECD test method guidance document.</p> <p>Degradation is calculated from the starting and final DOC concentrations. DOC concentrations were measured 24 times during the 35 day test.</p>
GLP:	no data
Remark:	<p>Hungate serum bottles were filled with water and the water was displaced with an inert gas mixture of carbon dioxide and methane. A 50 ml inoculum was injected into the serum bottle</p> <p>along with 100 mg of acetate and 25 mg of the test compound. Gas production was monitored and subsequently injections of the pure test compound were made with a microliter syringe as needed. Test substance was injected into the serum bottle to yield initial concentrations of 500 mg/l for the first six injections. Injections were increased to doses of 1000 mg/l in the 7th injection and thereafter. Daily additions of inorganic salt solution, plus acetate and the test material were made in a long term feeding acclimation study.</p>
Year:	1983
GLP:	no
Test substance:	isobutanol
Reliability:	Score=2, valid with restrictions, not all experimental details available
Reference:	Huels AG, 1983, Abschlussbericht CU-0405. Bestimmung der biologischen Abbaubarkeit von Isobutanol in Coupled Units Test, Marl, Germany.

3.6 BOD₅, COD OR RATIO BOD₅/COD

BOD₅

No data available

COD

No data available

Ratio BOD₅/COD:

No data available

3.7 BIOACCUMULATION

- (a) Type: Bioconcentration Factor (BCF)
Test substance: isobutanol
Method: Calculated using EPISUITE v.3.10 and BCFWIN v.2.14 with a log Kow of 0.79
Result: 3 L/kg
GLP: not applicable
Reliability: Score=2, valid with restrictions, calculation
Reference: EPISUITE v.3.10, U.S. Environmental Protection Agency (2000)

3.8 ADDITIONAL REMARKS

- (a) Type: Henry's Law constant
Test substance: isobutanol
Method: Calculated using water solubility 85,000 mg/L, vapor pressure (10.4 mm Hg), and molecular weight 74.12 g/mol.
Result: 1.19E-5 atm-m³/mol
GLP: Not applicable
Reliability: Score=2, valid with restrictions, calculation
Reference: Lyman, W.J., et al. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill NY.
- (b) POCP Value: 25 to 60
Remark: Photochemical Ozone Creation Potential (POCP) is a measure of the relative potential of a chemical to form ozone in the atmosphere. POCP is not measured directly but rather is developed from atmospheric and chemical mechanistic models. As a result, reported POCP values for a single chemical may vary considerably with atmospheric conditions including meteorology, amount of sunlight, and the concentration of nitrogen oxides and other volatile organic compounds already in and being newly emitted to the air.
A representative value of 37.5 (relative to ethene) is found in R. G. Derwent, et al., Photochemical Ozone Creation Potentials for Organic Compounds in Northwest Europe Calculated with a Master Chemical Mechanism, *Atmospheric Environment*, Vol. 32, No. 19, 1998.

4.0 ECOTOXICOLOGICAL DATA

4.1 ACUTE/PROLONGED TOXICITY TO FISH

- (a) Preferred value
- | | |
|---------------------|---|
| Type: | flow through |
| Species: | <i>Pimephales promelas</i> (fathead minnow) |
| Exposure Period: | 96 hour |
| Unit: | mg/l |
| Analyt. Monitoring: | yes |
| LC50: | 1430 |
| EC50: | 1430 |
| Method: | other, (USEPA) |
| Year: | 1984 |
| GLP: | no |
| Test substance: | isobutanol, purity >99% |
| Method: | Fathead minnows used in the tests were cultured from brood stock provided by the USEPA Environmental Research Laboratory-Duluth. Adults were maintained in a flow through system at 25 deg. C with a 16-h light/dark photoperiod. Organisms were fed frozen adult brine shrimp (<i>Artemia</i> sp.) Fry were fed freshly hatched brine shrimp nauplii three times daily until 24-h before test initiation. Fish were not fed during the test. Fish used in the toxicity tests were 30 d old and had a mean length of 19.7 $\bar{\Delta}$ 2.836 mm and a mean weight of 0.098 $\bar{\Delta}$ 0.0373 g. The loading rate was 0.389 g/l. The toxicity test was conducted using a flow-through exposure regime. The tank volume was 6.3 L. The control/dilution water was either dechlorinated laboratory water that was supplemented with minerals or filtered Lake Superior water. Total hardness was 47.8 $\bar{\Delta}$ 0.15 mg/l (as CaCO ₃), and alkalinity was 40.9 $\bar{\Delta}$ 0.11 mg/l (as CaCO ₃). |
- The test was initiated using 50 (30-d old) organisms, randomly distributed to each of five test concentrations, and an untreated control. The test was conducted with two replicates (25 fish per replicate) for each concentration tested and the control water. The purity of the test material and the test concentrations were analyzed by gas-liquid chromatography. Measurements of the test substance in the test concentrations were made five times during the exposure period. Nominal (and mean measured for each replicate) concentrations tested: 0 (0.0, 0.0) mg/l, 340 (209, 277) mg/l, 570 (432, 480) mg/l, 940 (717, 723) mg/l, 1570 (1271, 1225) mg/l, and 2620 (1900, 1747) mg/L. Mortality and signs of abnormal behavior were recorded at 0.5, 1, 2, 4, 6, 10, 24, 48, 72, and 96 hours. Effect concentrations were calculated based on mean measured concentrations.
- Result: The test temperature was 25.7 $\bar{\Delta}$ 0.11 degree C. The concentration of dissolved oxygen was 6.2 $\bar{\Delta}$ 0.057 mg/L; pH was pH was 7.58 $\bar{\Delta}$ 0.01 SU.
- Result: There was no control mortality. Affected fish lost equilibrium prior to death. The 96 hr EC50/LC50 (95% confidence limit) = 1430 (1370-1490) mg/L.
Control: No mortality observed, no signs of toxicity observed
340 mg/l: No mortality observed, no signs of toxicity observed
570 mg/l: No mortality observed, no signs of toxicity observed
940 mg/l: No mortality observed, no signs of toxicity observed

DATE: SEPTEMBER 2004

	1570 mg/l: 0 dead, 23 affected at 0.5 h, 1 dead, all affected at 1 h, 2 dead, 14 affected at 2 h, 3 dead, 10 affected at 4 h, 3 dead, 8 affected at 6 h, 4 dead, 5 affected at 10 h, 4, dead, 4 affected at 24 h, 4 dead, 4 affected at 48 h, 5 dead, 6 affected at 72 h, 5 dead, 5 affected at 96 h. This means that a total of 5 (of 50) fish exposed to 1570 mg/L were dead and a total of 5 fish remained affected at 96-hours. Exposed fish became less affected (observed lack of equilibrium) as the test progressed.
	2620 mg/l: 49 of 50 were dead by 24 h
Remark:	Authors noted that affected fish lost equilibrium prior to death. 96-h EC/LC50 and 95% CL = 1430 (1370-1490) mg/L
Reliability:	Score=1, valid without restrictions
References:	Brooke, L.T. et al., 1984. Acute Toxicities of Organic Chemicals to Fathead Minnows (<i>Pimephales promelas</i>). Vol. I. Center for Lake Superior Environmental Studies. University of Wisconsin-Superior.
(b) Value:	754 mg/L
Test substance:	Isobutanol
Remark:	An acute 96-h LC50 for fish was calculated using ECOSAR, from the USEPA. The preferred physical properties were used. The SAR for neutral organics was employed. 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, calculations
Reference:	EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).
(c) Value:	621 mg/L
Test substance:	n-butanol
Remark:	An acute 96-h LC50 for fish was calculated using ECOSAR, from the USEPA. The SAR for neutral organics was employed. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR. The physical properties used for the calculation were: molecular weight of 74.12 g/mol, melting point of -89.9 deg.C, water solubility of 77,000 mg/L, and log Kow of 0.88.
Reliability:	Score=2, valid with restrictions, calculations
Reference:	EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).
(d) Test Substance:	n-Butyl Alcohol
Method:	OECD 203, USEPA TSCA 40 CFR 797.1400
Year (guideline):	1992, 1994
Type (test type):	Static Fish Acute Toxicity Test
GLP:	Yes
Year (study performed):	1998
Species:	Fathead minnow (<i>Pimephales promelas</i>)
Analytical Monitoring:	Yes
Exposure Period:	96 Hours
Statistical Method:	(FT - ME)* Moving Average Method
Remark:	Test solutions were prepared by diluting a 50-mg/mL stock solution of n-butyl alcohol (99.9% purity) with moderately hard, filtered [0.2 mm] well water to nominal concentrations of 389, 648, 1080, 1800, and 3000 mg/L. Stock solution was also prepared with well water. Test vessels were 19-L glass aquaria containing approximately 15 L (12-cm depth) of test solution.

Two replicate test vessels were maintained for each treatment and control (dilution water) group. Vessels were covered and maintained in an environmental chamber for the test duration at 22 ± 2 °C with a 16-hour light: 8-hour dark photoperiod (381 lux).

Water samples for analytical verification were collected from each replicate of the control and treatments at test initiation and termination.

Dissolved oxygen exceeded 60% saturation and pH ranged from 7.8 to 8.6. Temperature ranged from 22.2 to 22.8 °C. Dilution water total organic carbon was <1 mg C/L. Total hardness, alkalinity, acidity, and specific conductance of dilution water were 132 mg/L as CaCO₃, 178 mg/L as CaCO₃, 20 mg/L as CaCO₃, and 310 mmhos/cm, respectively.

Fish were obtained from in-house cultures. Twenty minnows (10 per replicate) were exposed to each test concentration and control (dilution water). Average length of 10 control fish at test termination was 25 mm (range: 21 to 28 mm). Average weight (blotted dry) was 0.34 g (0.16 to 0.50 g). Loading was 0.23 g fish/L in test vessels.

Results: (FT - RS) 96-hour LC₅₀ was 1376 mg/L (95% CL: 1216 and 1587 mg/L) based on mean measured concentrations
 Reliability: (1) valid without restrictions
 Reference: Wong, D.C.L, P.B. Dorn, and J.P. Salanitro. 1998. Aquatic Toxicity of Four Oxy-Solvents. Equilon Enterprises, LLC Technical Information Record WTC-3520.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

(a) Preferred value (preferred aquatic invertebrate species)

Type: Static
 Species: *Daphnia magna Straus* (crustacea)
 Unit: mg/l
 Exposure Period: 48 hours
 EC50: 1300
 Method other: ASTM Methods (1984a,b) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians (Standard E-729-80) and Standard practice for conducting static acute toxicity tests on wastewaters with *Daphnia* (Standard D4229-84)
 Analytical Monitoring: Not Reported
 Year: 1984
 GLP: no
 Test substance: Isobutanol (2-methyl, 1-propanol), reagent grade
 Remark: *Daphnia* for stock cultures were obtained from the USEPA Duluth Laboratory. Ten <24-h old *D. magna Straus* were tested at 23 ± 1 °C in a series of five test concentrations (not specified) plus a clean water control. Tests were conducted in unchlorinated, carbon-filtered well water, aerated to saturation before use. Dilution water had a total hardness and alkalinity of 240 ± 10 and 230 ± 10 mg/L as CaCO₃, respectively, a pH of 8.0 ± 0.3 and a specific conductance of 360 ± 10 micromhos/cm².

Test concentrations were not measured. Daphnids were not fed. Tests were conducted in 250-mL beakers containing 200 mL of solution. A photo-

DATE: SEPTEMBER 2004

<p>Result: Remark: Reliability Reference:</p>	<p>period of 16 h light and 8 h dark with 15-min transition periods were used. All test vessels were covered with glass watch glasses. The estimated EC50 and 95% confidence limits were determined using probit analysis. 48-h EC50 (95% CL) = 1300 (1200-1400) mg/L This study was part of a collaborative testing effort with the US EPA's Environmental Research Laboratory at Duluth, MN. Score=2, valid with restrictions (absence of measured test concentrations) Elnabarawy MT, Welter AN, Robideau RR. 1986. relative sensitivity of three daphnid species to selected organic and inorganic chemicals. Environ Toxicol Chem 5: 393-398.</p>
<p>(b) (2) valid with restrictions (absence of measured test concentrations) Type: Species: Exposure period: Unit NOEC: EC50: Method: Analytical Monitoring: Year: GLP: Test substance: Remark:</p>	<p>static Daphnia pulex (crustacea) 48 hour(s) mg/L 1100 ASTM Methods (1984a,b) Standard practice for conducting acute toxicity tests fishes, macroinvertebrates, and amphibians (Standard E-729-80) and Standard practice for conducting static acute toxicity tests on wastewaters with Daphnia (Standard D4229-84). Not Reported 1984 no isobutanol (2-methyl, 1-propanol), reagent grade Daphnia for stock cultures were obtained from the USEPA Duluth Laboratory. Ten <24-h old D. pulex were tested at 23±1 °C in a series of five test concentrations (not specified) plus a clean water control. Tests were conducted in unchlorinated, carbon-filtered well water, aerated to saturation before use. Dilution water had a total hardness and alkalinity of 240±10 and 230±10 mg/L as CaCO₃, respectively, a pH of 8.0±0.3 and a specific conductance of 360±10 micromhos/cm². Test concentrations were not measured. Daphnids were not fed. Tests were conducted in 250-mL beakers containing 200 mL of solution. A photo-period of 16 h light and 8 h dark with 15-min transition periods were used. All test vessels were covered with glass watch glasses. The estimated EC50 and 95% confidence limits were determined using probit analysis. 48-h EC50 (95% CL) = 1100 (950-1200) mg/l This study was part of a collaborative testing effort with the US EPA's Environmental Research Laboratory at Duluth, MN. Score=2 valid with restrictions (absence of measured test concentrations) Elnabarawy MT, Welter AN, Robideau RR. 1986. Relative sensitivity of three daphnid species to selected organic and inorganic chemicals. Environ Toxicol Chem 5: 393-398.</p>
<p>(c) Type: Species: Exposure period: Unit EC50: Method:</p>	<p>static <i>Ceriodaphnia reticulata</i> (Crustacea) 48 hour(s) mg/L 1200 ASTM Methods (1984a,b) Standard practice for conducting acute toxicity tests fishes, macroinvertebrates, and amphibians (Standard E-729-80) and</p>

DATE: SEPTEMBER 2004

Analytical Monitoring:	Standard practice for conducting static acute toxicity tests on wastewaters with <i>Daphnia</i> (Standard D4229-84). Not Reported.
Year:	1984
GLP:	no
Test substance:	isobutanol (2-methyl, 1-propanol), reagent grade
Remark:	Cerio daphnia for stock cultures were obtained from the USEPA Duluth Laboratory. Ten <24-h old daphnids were tested at 23±1 °C in a series of five test concentrations (not specified) plus a clean water control. Tests were conducted in unchlorinated, carbon-filtered well water, aerated to saturation before use. Dilution water had a total hardness and alkalinity of 240±10 and 230±10 mg/L as CaCO ₃ , respectively, a pH of 8.0±0.3 and a specific conductance of 360±10 micromhos/cm ² . Test concentrations were not measured. Daphnids were not fed. Tests were conducted in 250-mL beakers containing 200 mL of solution. A photoperiod of 16 h light and 8 h dark with 15-min transition periods were used. All test vessels were covered with glass watch glasses. The estimated EC50 and 95% confidence limits were determined using probit analysis.
Result:	48-h EC50 (95% CL) = 1200 (1100-1300) mg/l
Remark:	This study was part of a collaborative testing effort with the US EPA's Environmental Research Laboratory at Duluth, MN.
Reliability:	Score=2 valid with restrictions (absence of measured test concentrations)
Reference:	Elnabarawy MT, Welter AN, Robideau RR. 1986. relative sensitivity of three daphnid species to selected organic and inorganic chemicals. Environ Toxicol Chem 5: 393-398.
(d) Value:	743 mg/L
Test substance:	isobutanol
Remark:	An acute 48-h LC50 for daphnids was calculated using ECOSAR, from the USEPA. The preferred physical properties were used. The SAR for neutral organics was employed. 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, calculation
Reference:	EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).
(e) Test Substance:	n-Butyl Alcohol
Method:	OECD 202, USEPA TSCA 40 CFR 797.1300
Year (guideline):	1984, 1994
Type (test type):	Static Daphnid Acute Toxicity Test
GLP:	Yes
Year (study performed):	1998
Species:	Water flea (<i>Daphnia magna</i>)
Analytical Monitoring:	Yes
Exposure Period:	48 Hours
Statistical Method:	(FT – ME)* Binomial probability with non-linear interpolation
Test Conditions:	(FT – TC)
Remark:	Test solutions were prepared by diluting a 50-mg/mL stock solution of n-butyl alcohol (99.9% purity) with moderately hard, filtered [0.2 mm] well water to nominal concentrations of 156, 259, 432, 720, 1200, and 2000 mg/L. Stock solution was also prepared with well water. Test vessels were 250-mL beakers containing approximately 200 mL (7.8-cm depth) of test solution. Two replicate test vessels were maintained for each treatment and

control (dilution water) group. Vessels were covered to prevent evaporation and placed in a water bath at 20±1°C with a 16-hour light: 8-hour dark photoperiod (391 lux).

Water samples for analytical verification were collected from each replicate of the control and treatments at test initiation and termination.

Dissolved oxygen exceeded 60% saturation and pH ranged from 8.2 to 8.5. Temperature ranged from 19.4 to 19.7 °C. Dilution water total organic carbon was <1 mg C/L. Total hardness, alkalinity, and specific conductance of dilution water were 128 mg/L as CaCO₃, 180 mg/L as CaCO₃, and 300 mmhos/cm, respectively.

Daphnids were obtained from in-house cultures. Adult organisms were held for at least 16 days prior to collection of neonates for testing. Twenty daphnids (10 per replicate) <24 hours old were exposed to each test concentration and control (dilution water).

Results: (FT - RS) 48-hour EC₅₀ was 1328 mg/L (95% CL: 1123 and 1925 mg/L) based on mean measured concentrations.

Some organisms appeared lethargic in the 675 mg/L test solution after 48 hours and in the 1123 mg/L treatment after 21, 24, and 48 hours. All surviving organisms exposed to 1925 mg/L appeared lethargic at the 21 and 24-hour observations.

Reliability: (1) valid without restrictions

Reference: Wong, D.C.L, P.B. Dorn, and J.P. Salanitro. 1998. Aquatic Toxicity of Four Oxy-Solvents. Equilon Enterprises, LLC Technical Information Record WTC-3520.

(f) Value: 615 mg/L
Test substance: n-butanol
Remark: An acute 48-h EC₅₀ for daphnids was calculated using ECOSAR, from the USEPA.

The SAR for neutral organics was employed. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR. The physical properties used for the calculation were: molecular weight of 74.12 g/mol, melting point of -89.9 deg.C, water solubility of 77,000 mg/L, and log Kow of 0.88.

Reliability: Score=2, valid with restrictions, calculations

Reference: EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).

4.3 TOXICITY TO AQUATIC PLANTS e.g. Algae

(a) Remark: No algal data available for isobutanol, however, there are acute algae toxicity data for the structurally similar n-butanol
Test Substance: n-Butyl Alcohol
Species: *Selenastrum capricornutum* (Freshwater green algae)
Method: OECD 201, USEPA TSCA 40 CFR 797.1050
Year (guideline): 1984, 1994
Test (test type): Static Algal Toxicity Test
GLP: yes
Year (study performed): 1998

Analyt. Monitoring: yes
 Exposure period: 96 hour(s)
 Statistical Method: (FT -ME)*Linear interpolation for EC values, Dunnett's test for NOAEC
 Test Conditions: (FT – TC) Test solutions were prepared by diluting a 50-mg/mL stock solution of n-butyl alcohol (99.9% purity) with laboratory-prepared algal nutrient medium to nominal concentrations of 125, 250, 500, 1000, and 2000 mg/L. Stock solution was also prepared with nutrient medium. Test vessels were sterile, 250-mL Erlenmeyer flasks plugged with foam stoppers and contained 100 mL of test or control (nutrient medium) solution. Vessels were continuously shaken mechanically at 100 rpm. Three replicate vessels were maintained for each treatment and control group. Initial cell density was 1.0×10^4 cells/mL (nominal). Samples were collected from each replicate test vessel at each 24-hour interval and held at 4°C until cell density measurement. Cell counts were obtained using an electronic particle counter (Coulter Electronics, Inc.).

Cell densities were used to calculate growth inhibition values and effects concentrations (EC10, EC50, and EC90) relative to the control. Algal growth inhibition was differentiated as algicidal or algistatic effects at test termination by subculturing test solutions with maximally inhibited growth to fresh nutrient medium for a 9-day recovery period.

Water samples for analytical verification were collected at test initiation from the preparation vessels of each treatment and the control. Samples collected at test termination were a composite of the replicates for each treatment and the control and were filtered to remove the algae prior to analysis.

Temperature ranged from 23.2 to 25.3 °C. Light was continuous at 4240 to 4568 lux. Measurements of pH 7.4 at test initiation and ranged from 6.8 to 7.7 at 96 hours.

Original algal cultures were obtained from UTEX – The Culture Collection of Algae at the University of Texas at Austin and were maintained in culture medium for at least two weeks prior to testing.

Based on Day 0 measured n-butyl alcohol concentrations:

96-hour EC₁₀ = 134 mg/L (95% CL: 124 - 167 mg/L)

96-hour EC₅₀ = 225 mg/L (95% CL: 204 - 246 mg/L)

96-hour EC₉₀ = 717 mg/L (95% CL: 586 - 809 mg/L)

96-hour growth rate inhibition:

<u>Day 0 Measured Concentration (mg/L)</u>	<u>96-hour % Inhibition</u>	<u>96-hour Cell Density</u>
Control	--	4,206,362
129	7.7	3,883,813
241	57	1,808,913*
491	83	732,225*
1010	100	15,521*
* 1980	100	15,754*
I		
n		

Indicates significant difference from control using Dunnett's test ($p \leq 0.05$)

Changes in cell density indicated that exponential growth occurred in the control replicates. The coefficient of variation for the control replicates was 8.5%.

Algal cells in 1980 mg/L (2000 mg/L nominal) resumed normal growth after 9 days. Effects on algal growth were considered algistatic.

Measured concentrations of test solutions at test initiation ranged from 97 to 103% of nominal values. Measured concentrations after 96 hours ranged from <LOQ to 73% of nominal.

n-Butyl Alcohol concentrations in test chambers were determined using a Hewlett-Packard Model 5890 Gas Chromatograph with flame ionization detector.

- Reliability: (1) Reliable without restriction. OECD endpoints were not determined
Reference: Wong, D.C.L, P.B. Dorn, and J.P. Salanitro. 1998. Aquatic Toxicity of Four Oxy-Solvents. Equilon Enterprises, LLC Technical Information Record WTC-3520.
- (b) Value: 433 mg/L
Test Substance: isobutanol
Test Species: Green Algae
Remark: An acute 96-h EC50 for green algae was calculated using ECOSAR, from the USEPA. The preferred physical properties were used. The SAR for neutral organic was employed. 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, calculation
Reference: EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency (2000).
- (c) Test substance: isobutanol
Test species: *Scenedesmus quadricauda* (Green Algae)
Test method: Cell Multiplication Inhibition Test
Test duration: 8 days
GLP: no
Test results: 350 mg/l
Reliability: Score=3, not valid due to non-standard study duration
Reference: Bringmann G., Kühn R. Comparison of the Toxicity Thresholds of Water Pollutants to Bacteria, Algae and Protozoa in the Cell Multiplication Inhibition Test. *Water Research* 14:231-241. 1980.
- (d) Test substance: isobutanol
Test species: *Microcystis aeruginosa* (Blue-Green Algae)
Test duration: 8 days
Test method: Cell Multiplication Inhibition Test
GLP: no
Test results: 290 mg/l
Reliability: Score=3, not valid due to non-standard study duration

DATE: SEPTEMBER 2004

Reference:	Bringmann G., Kühn R. Comparison of the Toxicity Thresholds of Water Pollutants to Bacteria, Algae and Protozoa in the Cell Multiplication Inhibition Test. <i>Water Research</i> 14:231-241. 1980.
(e) Test substance:	isobutanol
Test species:	<i>Scenedesmus subspicatus</i> Green Algae)
Test duration:	48 hours
Test method:	Cell Multiplication Inhibition Test
GLP:	no
Test results:	290 mg/l
Reliability:	(2), valid with care, full experimental details not available
Reference:	Kuhn R, Pattard M. 1990. Results of the harmful effects of water pollutants to green algae (<i>Scenedesmus subspicatus</i>) in the cell multiplication inhibition test. <i>Water Res.</i> 24: 31-38.
(f) Value:	361 mg/L
Test Substance:	n-butanol
Remark:	An acute 96-h EC50 for green algae was calculated using ECOSAR, from the USEPA. The preferred physical properties were used. The SAR for esters was employed. The structure was determined from the CAS RN as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability:	(2) valid with care, calculation
Reference:	EPA's ECOSAR model (v. 0.99f). EPISUITE v.3.10, U.S. Environmental Protection Agency, April (2001).

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

5.0 TOXICITY

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a) Preferred value

Type: LD50
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 LD₅₀. 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 CO₂ 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 LD₅₀ 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

DATE: SEPTEMBER 2004

		and the uterus was saved in order to verify this condition (since the animals are ordered to be nonpregnant).
Year:		1993
GLP:		Yes
Test substance:		Isobutanol (99.9% purity by capillary GC, GC/MS and NMR used to confirm identity)
Remark:		<p>In preliminary testing, 1 female rat was dosed with 2000 mg/kg of isobutanol 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 LD₅₀ 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 isobutanol (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 evaluation). 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.</p> <p>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, decidualoma 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) LD₅₀ was > 2830 mg/kg bw; 0 of 3 died. The female rat oral (fasted) LD₅₀ was 3350 (2860 to 3920) mg/kg bw. Microscopic kidney lesions were evident but probably not related to treatment.</p>
Reliability:		Score = 1, GLP guideline study
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
(b)	Type:	LD50
	Species:	rat
	Value:	3100 mg/kg
	Method:	
	Year:	1983

	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	Kushneva, V.S. et al., Gig. Tr. Prof. Zabol. 1, 46-47 (1983). Zit. nach: Environmental Health Criteria 65, Isobutanol, World Health Organization, Geneva (1987) cited in IUCLID (2000).
(c)	Type:	LD50
	Species:	rat
	Value:	2460 mg/kg
	Method:	
	Year:	1954
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 2, collection of data
	Reference:	Smyth et al., AMA Arch. Ind. Hyg. Occup. Med. 10: 61-68, (1954) cited in IUCLID (2000).
(d)	Type:	LD50
	Species:	rat
	Value:	2650 mg/kg
	Method:	
	Year:	1969
	GLP:	
	Test substance:	Isobutanol
	Remark:	Male rats; LD50-range: 1790-3990 mg/kg. Fatally poisoned rats died within 18 hours and exhibited hyperaemia of the liver, and fatty infiltration, swelling, and necrosis of the kidneys.
	Reliability:	Score = 4, original reference not available
	Reference:	Purchase I.H.F.: S. Afr. Med. J., 54, 795-798, (1969); cited in BG Chemie (ed.): 2-Methylpropanol-1, in: Toxicological Evaluations 1 - Potential Health Hazards of Existing Chemicals, Springer Verlag, Berlin, pp. 43-57, (1990) cited in IUCLID (2000).
(e)	Type:	LD50
	Species:	rat
	Value:	2740 mg/kg
	Method:	
	Year:	1951
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	TSCATS: OTS 0510383, Doc. I.D.: 878216455, 11.23.1951, Union Carbide Corp. cited in IUCLID (2000).
(f)	Type:	LD50
	Species:	mouse
	Value:	3500 mg/kg
	Method:	

DATE: SEPTEMBER 2004

	Year:	1983
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	Kushneva, V.S. et al., Gig. Tr. Prof. Zabol. 1, 46-47 (1983). Zit. nach: Environmental Health Criteria 65, Isobutanol, World Health Organization, Geneva (1987) cited in IUCLID (2000).
(g)	Type:	LD50
	Species:	rabbit
	Value:	3040 mg/kg
	Method:	
	Year:	1972
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	Munch J.C.: Ind. Med., 41, 31-33, (1972); cited in BG Chemie (ed.): 2-Methylpropanol-1, in: Toxicological Evaluations 1 -Potential Health Hazards of Existing Chemicals, Springer Verlag, Berlin, pp. 43-57, (1990) cited in IUCLID (2000).
(h)	Type:	LD _{low}
	Species:	rabbit
	Value:	3750 mg/kg
	Method:	
	Year:	1978
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	US DHEW, US Department of Health, Education and Welfare, Washington DC, (1978); cited in WHO: Environmental Health Criteria 65, WHO, Geneva, pp. 93 ff., (1987) cited in IUCLID (2000).
(i)	Type:	LD _{low}
	Species:	rabbit
	Value:	3000 mg/kg
	Method:	
	Year:	1925
	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	Munch, J.C. & Schwartze, E.W., J. Lab. Clin. Med. 10, 985-996 (1925). Zit. nach: Toxikologische Bewertung, Nr. 96, 2-Methylpropanol-1, Berufsgenossenschaft der Chemischen Industrie (1988) cited in IUCLID (2000).
(j)	Type:	ND50 "Narcotic Dose"
	Species:	rabbit
	Value:	1404 mg/kg
	Method:	

Year: 1972
 GLP:
 Test substance: Isobutanol
 Remark: ND50 was the dose, which caused mild narcosis in half of the animals (as shown by stupor, lying on the side or stomach, short-term resumption of movement and standing up on manual compression or stimulation). At a higher not specified dose, rabbits exhibited loss of corneal reflexes, nystagmus, bradycardia, and dyspnea.

Reliability: Score = 4, original reference not available
 Reference: Munch J.C.: *IMS Ind. Med. Surg.*, 41, 31-33, (1972); cited in BG Chemie (ed.): *2-Methylpropanol-1*, in: *Toxicological Evaluations 1 - Potential Health Hazards of Existing Chemicals*, Springer Verlag, Berlin, pp. 43-57, (1990) cited in IUCALID (2000).

5.1.2 ACUTE INHALATION TOXICITY

- (a) Preferred result
- 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/m³)
 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, GLP guideline study

DATE: SEPTEMBER 2004

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.
(b) Type:	LClow
Species:	rat
Value:	6-Hour saturated static exposure - Males: 0 of 5 died, Females: 0 of 5 died.
Method:	The objective of this study was to assess the acute inhalation toxicity of isobutanol. There are no specific guidelines for the acute inhalation test. Rat (Harlan Sprague Dawley) body weights were within +/- 20% of the group mean for each sex. For the inhalation test, the body weight range on the day of dosing was 286 to 298 g for males and 209 to 218 g for females. A total of 5 male and 5 female rats were used in the inhalation toxicity test. The animals were acclimated for at least 5 days before exposure. 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. A substantially saturated vapor was produced by enclosing 110 g of isobutanol in a sealed 120 liter animal chamber for approximately 15 hours under static conditions. In order to aid in the distribution of the vapor, a mixing fan periodically agitated the chamber atmosphere. No analysis of the exposure concentration was performed. Oxygen was added as needed to the chamber in order to maintain a chamber oxygen content of approximately 20%. The average temperature and humidity in the chamber during the exposure period were approximately 22°C and 92%, respectively. No analysis was made of the chamber atmosphere for the concentration of the test substance. Five male and 5 female rats were placed into the chamber and sealed inside for the 6-hour exposure period. Dosed rats were observed frequently during the exposure for signs of toxicity 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. At 14 days, all animals were sacrificed using methoxyflurane and necropsied. Following a 6-hour inhalation exposure, pseudopregnancy was observed in 1 female rat and the uterus was saved. Histology was performed on the uterus to verify this finding and also to compare results with those from the 1 female rat in the peroral test that appeared to be pregnant.
Year:	1993
GLP:	Yes
Test substance:	Isobutanol (99.9% purity by capillary GC; GC/MS and NMR used to confirm identity)
Remark:	Exposure to a statically-generated, substantially saturated vapor did not produce deaths in any of 5 male or 5 female rats during or following the 6-hour test. Signs of toxicity observed during exposure included hypoactivity, lacrimation, narcosis, prostration, abnormal breathing (short, shallow breaths) and wetness of the periocular fur. Prostration, narcosis and negative reflexes (surface righting and toe and tail pinch) were also observed following exposure. All animals recovered by 1 day. Most animals had a consistent weight gain. One female rat exhibited a slight weight loss by 7 days but partially recovered within 14 days. Necropsy revealed red or brown foci on the lungs. One female rat had several focal implants in the left uterine horn (pseudopregnancy). The uterus from the 1 female rat that exhibited

DATE: SEPTEMBER 2004

pseudopregnancy was saved and examined microscopically. Deciduoma of pseudopregnancy were also apparent in this animal as in the rat from the peroral test. 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 oral study) had pseudopregnancy due to cervical stimulation from the vaginal swabbing procedure.

- Reliability: Score = 1, GLP, accepted scientific method
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
- (c) Type: LC50
Species: rat
Value: 19.2 mg/L (6336 ppm)
Method: Four hour exposure
Year: 1983
GLP:
Test substance: Isobutanol
Remark: Symptoms of toxic effects: Irritation of the airways, decreases in the activity of the central nervous system; decrease of leukocytes in the bone marrow; reduced lactate level in the blood; retarded elimination of bromophthalein from the blood; dystrophic changes of the hepatocytes and olfactory cells.
Reliability: Score = 4, original reference not available
Reference: Kushneva V.S. et al.: Gig. Tr. Prof. Zabol., 1, 46-47, (1983); cited in WHO: Environmental Health Criteria 65, WHO, Geneva, pp. 93 ff., (1987) as cited in IUCLID.
- (d) Type: LC50
Species: rat
Value: >6.5 mg/L (2145 ppm)
Method: Four hour exposure.
Year: 1979
GLP:
Test substance: Isobutanol (purity 99.5%)
Remark: All 10 males and 10 female Sprague-Dawley rats survived 4-hr exposure to 6.5 mg/L isobutanol vapors. They exhibited no signs of toxicity throughout the exposure and the 14-day post-exposure period.
Reliability: Score = 4, original reference not available
Reference: BASF AG (a), Department of Toxicology: "Bericht ueber die Bestimmung der akuten Inhalationstoxizitaet LC50 von i-Butanol bei 4stuendiger Exposition an Sprague-Dawley-Ratten", unpublished report, (78/306), 03.12.1979 as cited in IUCLID.
- (e) Type: LC_{low}
Species: rat
Value: 8 mg/L (2640 ppm)
Method: Four hour exposure
Year: 1978

	GLP:	
	Test substance:	Isobutanol
	Remark:	
	Reliability:	Score = 4, original reference not available
	Reference:	US DHEW, US Department of Health, Education and Welfare, Washington DC, (1978); cited in WHO: Environmental Health Criteria 65, WHO, Geneva, pp. 93 ff., (1987) as cited in IUCLID.
(f)	Type:	LC _{low}
	Species:	rat
	Value:	8000 ppm (24,240 mg/m ³)
	Method:	Four hour exposure.
	Year:	1954
	GLP:	
	Test substance:	Isobutanol
	Reliability:	Score = 2, collection of data
	Remark:	
	Reference:	Archives of Industrial Hygiene and Occupational Medicine. (Chicago, IL) V.10,61,1954. as cited in IUCLID.
(g)	Type:	LC _{low}
	Species:	rat
	Value:	
	Method:	two or four hour exposure
	Year:	1954
	GLP:	
	Test substance:	Isobutanol
	Remark:	Exposure to a concentrated vapor of isobutanol for 2 hr at maximum did not induce any lethality; a 4 hr exposure to nominal 8000 ppm (ca. 24.6 mg/L) resulted in the death of 2/6 animals.
	Reliability:	Score = 2, collection of data
	Reference:	Smyth H.F. Jr. et al.: Arch. Ind. Hyg. Occup. Med., 10, 61-68, (1954) as cited in IUCLID.
(h)	Type:	LC _{low}
	Species:	rat
	Value:	
	Method:	seven hour exposure according to method of Smyth, et al., Am. Ind. Hyg. Assoc. J. 23:95-107, 1962
	Year:	1978
	GLP:	
	Test substance:	Isobutanol
	Remark:	Rats were exposed to an atmosphere enriched with the test substance at 20°C. 12/12 rats survived exposure for 3 hours but 1 of 6 rats died after 7 hours inhalative isobutanol exposure, showing grey coloration of the liver at necropsy. Signs of toxicity during exposure included eyelid closure, watery nasal secretion, reduced pain reaction and narcosis on the day of treatment. Gross pathology of rats killed after a 14-day post-observation period did not reveal any treatment related effects.
	Reliability:	Score = 4, original reference not available

DATE: SEPTEMBER 2004

Reference:	BASF AG (b), Department of Toxicology: "Bericht ueber die Pruefung der akuten Inhalationsgefahr (akutes Inhalationsrisiko) von i-Butanol, Prod Nr. 00902 an Sprague-Dawley-Ratten", unpublished report, (78/306), 03.12.1979 BASF AG, Department of Toxicology: Unpublished report (77/668), 10.27.1978 as cited in IUCLID.
(i) Type:	LC _{low}
Species:	rat
Value:	
Method:	2 and 4 hour exposures to substantially saturated vapors
Year:	1953
GLP:	
Test substance:	Isobutanol
Remark:	A 2 hr exposure to a "substantially saturated vapor" (ca. 14,000 ppm or 43 mg/L) was lethal to 0/6 females. A 4 hr exposure to a "substantially saturated vapor" (ca. 14,000 ppm or 43 mg/L) was lethal to 6/6 females. A 4 hr exposure to 8000 ppm (ca. 24.7 mg/L) of a 1946 sample was not lethal to males. Exposure to a 1953 sample killed 1/6 males and 0/6 females.
Reliability:	Score = 4, original reference not available
Reference:	TSCATS: OTS 0510381, Doc. I.D.: 878216453, 11.17.1953, Union Carbide Corp. as cited in IUCLID.
(j) Type:	LC50
Species:	mouse
Value:	15.5 mg/L
Method:	
Year:	1983
GLP:	
Test substance:	Isobutanol
Remark:	
Reliability:	Score = 4, original reference not available
Reference:	Kushneva V.S. et al.: Gig. Tr. Prof. Zabol., 1, 46-47, (1983); cited in WHO: Environmental Health Criteria 65, WHO, Geneva, pp. 93 ff., (1987) as cited in IUCLID.
(k) Type:	LC50
Species:	rabbit
Value:	26.25 mg/L
Method:	4 hours
Year:	1953
GLP:	
Test substance:	Isobutanol
Remark:	Symptoms of toxic effects: Irritation of the airways, decrease in the activity of the central nervous system, decrease in leukocytes in the bone marrow, reduced lactate level in the blood, retarded elimination of bromophthalein from the blood, dystrophic changes of the hepatocytes and olfactory cells.
Reliability:	Score = 4, original reference not available

DATE: SEPTEMBER 2004

Reference:	Kushneva V.S. et al.: Gig. Tr. Prof. Zabol., 1, 46-47, (1983); cited in WHO: Environmental Health Criteria 65, WHO, Geneva, pp. 93 ff., (1987) as cited in IUCLID.
(l) Type:	LC50
Species:	guinea pig
Value:	19.9 mg/L
Method:	4 hours
Year:	1983
GLP:	
Test substance:	Isobutanol
Remark:	
Reliability:	Score = 4, original reference not available
Reference:	Kushneva, V.S. et al., Gig. Tr. Prof. Zabol. 1, 46-47 (1983). Zit. nach: Environmental Health Criteria 65, Isobutanol, World Health Organization, Geneva (1987) as cited in IUCLID.

5.1.3 ACUTE DERMAL TOXICITY

(a) Preferred value	
Type:	LD50
Species:	rabbit
Value:	Males: LD ₅₀ > 2000 mg/kg - 0 of 3 died Females: LD ₅₀ = 2460 (1790 to 3390) mg/kg
Method:	Conducted in accordance with EPA (TSCA) Health Effects Testing Guidelines 40 CFR Part 798 (Subpart B, Sections 798.1100:acute dermal toxicity) and 1987 OECD Guidelines for Testing of Chemicals (Section 4: Health Effects; 402:acute dermal toxicity). Male and female New Zealand White rabbits were received from Hazleton Research Products, Inc. (Denver, PA). Rabbits were ordered to be between 2.0 and 2.3 kg (designated by the supplier to be approximately 12 to 14 weeks of age). The females were nulliparous and nonpregnant. 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. In addition, the rabbits were examined and weighed twice prior to dosing. Cage-side observations and mortality checks were conducted at least once daily. Animals considered unacceptable for the study, based on the clinical signs or body weights were rejected for use on this study. Only rabbits demonstrating weight gain were used. Rabbits weighing between 2.0 and 3.0 kg (approximately 13 to 18 weeks of age) were considered suitable for the definitive tests. The body weight range (on day of dosing) for males was 2.4 to 3.0 kg. For females, the body weight range was 2.4 to 3.4 kg. A total of 9 males and 21 females were used for the definitive rabbit tests. The fur was removed from the entire trunk of each rabbit using veterinary clippers at least 1 day before application of the test substance. As necessary, the rabbit skin was carefully trimmed (to remove excess re-growth of fur) up to the day before dosing. Only animals with an intact and normal epidermis were used in the study. A double layer of gauze sheeting was wrapped around the trunk and secured with adhesive tape. Polyethylene sheeting was then wrapped around the trunk over the gauze. To secure the polyethylene, plastic ties or rubber bands were added (at the ends of the trunk). The test substance had a tendency to adhere to the inside of the syringe during dosing causing the plunger to stick. Therefore, in order to minimize the potential for exposure by spraying, the undiluted test substance was applied

DATE: SEPTEMBER 2004

under the plastic wraps for most animals, covering as large a skin area as possible. The area of skin covered/dose level could not be measured except for 1 rabbit at 1.0 g/kg for which the dose was applied directly to the skin prior to wrapping. The amount of test substance applied was recorded for each animal. The sheeting was then protected from removal or tearing by wrapping the rabbit trunk with VETRAPH[®] bandaging tape (Myers, et al., 1989). The ends of the VETRAPH[®] were secured. After the 24-hour contact period, all coverings were removed. In the definitive percutaneous toxicity test, 5 female rabbits were included on each of several dose levels in order to determine an LD₅₀. Three male rabbits were included on an intermediate dose level for comparison. One female rabbit was used for preliminary percutaneous toxicity testing. For individual animals, the dose volume was adjusted according to body weight. Treated rabbits were observed frequently for signs of toxic effect 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 ear vein injection using Euthanasia-6 Solution (Veterinarian Laboratories Inc., Lenexa, KS). Necropsies were performed on all animals that died or were sacrificed. The following tissues (unless excessively autolyzed) were collected and retained in 10% neutral buffered formalin: kidneys, urinary bladder, liver, sciatic nerve and spleen. Because of possible lung damage as based on clinical signs, these tissues were also saved from selected animals.

Year 1993
GLP: Yes
Test substance: Isobutanol (99.9% purity by capillary GC, GC/MS and NMR used to confirm identity)
Remark: One rabbit was dosed with 8.0 g/kg of isobutanol in preliminary percutaneous toxicity testing (24-hour occluded contact) and died. In the definitive percutaneous toxicity test, the LD₅₀ for female rabbits was 2460 mg/kg of undiluted isobutanol. None of 3 male rabbits died following application of 2000 mg/kg (a dose that produced 1 of 5 female deaths); signs were noted. The amount of test substance/dose area covered was 20 mg/cm² for female rabbits at 1000 mg/kg. Dermal reactions included erythema, edema, necrosis, ecchymoses (on 2), fissuring, ulceration (on 1), desquamation, scabs and alopecia. Signs of toxicity observed included sluggishness, lacrimation (in 1), transient tremors (in 1), prostration, an unsteady gait (in 1), abnormal breathing (slow and/or labored), red eyes (conjunctivae, iris and/or nictitating membrane) and wetness of the periurogenital fur (of 1). For 1 to 2 days, 1 rabbit held its head abnormally low with its eyes directed upward; this animal eventually returned to normal. Several animals exhibited a weight loss by 7 days, but most recovered by 14 days. Deaths occurred within 3 hours to 1 day. Most survivors recovered at 3 hours to 1 day. One female (at 2000 mg/kg) recovered within 5 days. Gross pathologic evaluation of animals that died revealed red patches or areas on the lungs, dark red lungs (in 1), discolored and/or mottled livers (tan or darkened), gas-filled (characterized by bubbles) intestines (in 2), darkened spleens (in 2), dark red foci on 1 spleen, enlarged adrenals (in 1), kidneys with a pitted surface (in 1) and a trace amount of blood in the urine of 1 (positive by HEMASTIX. Reagent Strips). Necropsy of survivors revealed red to dark red patches or areas on the lungs (in 2), gas-filled intestines (in 1), 1 mottled dark maroon and light tan spleen, kidneys with a pitted surface (in 1) and tan kidneys (in 2). Isobutanol was moderately toxic following single 24-hour occluded contact with rabbit skin.

DATE: SEPTEMBER 2004

Reliability:	Score = 1, GLP guideline study
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.
(b) Type:	LD50
Species:	rabbit
Value:	4240 mg/kg
Method:	
Year	1954
GLP:	
Test substance:	Isobutanol
Remark:	
Reliability:	Score = 2, collection of data
Reference:	Smyth et al., AMA Arch. Ind. Hyg. Occup. Med. 10: 61-68, (1954) as cited in IUCLID.
(c) Type:	LD50
Species:	rabbit
Value:	3400 mg/kg
Method:	occlusive 24 hour exposure to undiluted test substance
Year	1944
GLP:	
Test substance:	Isobutanol
Remark:	Original LD50 value: 4.24 (2.52 to 7.12) ml/kg
Reliability:	Score = 2, collection of data
Reference:	Smyth H.F. Jr. et al.: AMA Arch. Ind. Hyg. Occup. Med., 10, 61-68, (1954) as cited in IUCLID.

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

(a) Preferred value	
Species:	rabbit
Result:	Minor to moderate erythema and edema on 6 of 6 rabbits, superficial necrosis on 2, ecchymoses on 1, fissuring on 1, desquamation on 4 and alopecia on 2 from 0.5 ml. Two rabbits had a normal appearance within 14 days; minor irritation persisted on the remaining 4 rabbits.
Classification:	Application of 0.5 ml of test substance for 4 hours to occluded rabbit skin resulted in minor to moderate irritation.
Method other:	Testing was conducted in accordance with EPA (TSCA) Health Effects Testing Guidelines 40 CFR Part 798 (Subpart E, Sections 798.4470:primary dermal irritation) and 1987 OECD Guidelines for Testing of Chemicals (Section 4: Health Effects; 404:acute dermal irritation/corrosion). Male and female New Zealand White rabbits were received from Hazleton Research Products, Inc. (Denver, PA). Rabbits were ordered to be between 2.0 and 2.3 kg (designated by the supplier to be approximately 12 to 14 weeks of age). The females were nulliparous and nonpregnant. 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. In addition, the rabbits were examined and weighed twice prior to dosing. Cage-side observations and mortality checks were conducted at least once daily. Animals considered unacceptable for the

study, based on the clinical signs or body weights were rejected for use on this study. Only rabbits demonstrating weight gain were used. Rabbits weighing between 2.0 and 3.0 kg (approximately 13 to 18 weeks of age) were considered suitable for the definitive tests. The body weight range (on day of dosing) for males was 2.4 to 3.0 kg. For females, the body weight range was 2.4 to 3.4 kg. The fur was removed from the dorsal area of the trunk of each rabbit using veterinary clippers a few days before dosing and the dose area was trimmed carefully (avoiding skin abrasion), as necessary, up to the day before application of the test substance. The test substance was applied to each of 6 rabbits (3 males, 3 females). Readings were made at 1,24,48 and 72 hours and at 7 and 14 days, after the end of the contact period according to the system of Draize. A 1-inch square gauze patch was placed over 1 intact (nonabraded) site/rabbit and secured by adhesive tape. A volume of 0.5 ml was then applied under the patch. Polyethylene sheeting was placed loosely around the trunk and secured. The animal was placed in a restraining device for the 4-hour contact period after which the coverings and as much excess test substance as possible were removed. All rabbits were sacrificed at 14 days (ear vein injection using Euthanasia-6 Solution).

Year: 1993
 GLP: Yes
 Test substance: Isobutanol (99.9% purity by capillary GC, GC/MS and NMR used to confirm identity).
 Remark: Application of 0.5 ml of isobutanol to covered rabbit skin for a 4-hour contact period produced minor to moderate erythema and edema on 6 of 6 rabbits) within 1 day. One rabbit had a light brown discoloration on the dose site at 1 hour. Superficial necrosis developed on this animal by 1 day; another rabbit had superficial necrosis at 7 days. Ecchymoses were apparent on 1 animal within 1 day. At 7 days, fissuring was observed on 1 animal. Four rabbits had desquamation at this time. By 14 days, alopecia was observed on 2 rabbits. Erythema and edema subsided on 5 of 6 rabbits within 14 days; minor erythema and edema persisted on 1 rabbit. Two rabbits had a normal appearance at this time.
 Reliability: Score = 1, GLP guideline study
 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.

5.2.2 EYE IRRITATION/CORROSION

(a) Preferred value
 Species: rabbit
 Result: Minor to moderate corneal injury in 2 of 2 rabbit eyes (including vascularization in 1), iritis in 2, severe conjunctival irritation in 2 (including hemorrhages of the nictitating membrane, severe swelling and a pus-like discharge), alopecia of the periocular area in 2 (with a small scab on 1) from 0.1 ml. Minor conjunctival redness apparent at 21 days.
 Classification: severe eye irritant
 Method: Testing was conducted in accordance with EPA (TSCA) Health Effects Testing Guidelines 40 CFR Part 798 (Subpart E, Sections 798.4500:primaryeye irritation) and 1987 OECD Guidelines for Testing of Chemicals (Section 4: Health Effects; 405:acute eye irritation/corrosion). Male and female New Zealand White rabbits were received from Hazleton Research Products, Inc. (Denver, PA). Rabbits were ordered to be between 2.0 and 2.3 kg (designated by the supplier to be approximately 12 to 14

weeks of age). The females were nulliparous and nonpregnant. 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. In addition, the rabbits were examined and weighed twice prior to dosing. Cage-side observations and mortality checks were conducted at least once daily. Animals considered unacceptable for the study, based on the clinical signs or body weights were rejected for use on this study. Only rabbits demonstrating weight gain were used. Rabbits weighing between 2.0 and 3.0 kg (approximately 13 to 18 weeks of age) were considered suitable for the definitive tests. The body weight range (on day of dosing) for males was 2.4 to 3.0 kg. For females, the body weight range was 2.4 to 3.4 kg. Both eyes of each rabbit to be dosed were examined, using fluorescein stain, within 24 hours before application. If any preexisting eye injury was apparent, the rabbit was rejected for use in the test. A volume of 0.1 ml of test substance was placed into the conjunctival sac of 1 eye/rabbit. The other eye of each animal served as the control. A total of 2 rabbits (1 male and 1 female) were first dosed because of the potential for the test substance to produce severe ocular irritation. Eye examinations were made at 1,24,48,52.5 hours and at 7,9,14,15 and 21 days following instillation. Readings were not made at 72 hours because of a lost workday resulting from severe weather conditions. However, in anticipation of the lost workday, an additional reading was made in the afternoon of the second day following dose administration. Fluorescein staining was performed at 1 day and each subsequent examination day. Grading and scoring were performed by the system of Draize. All rabbits were sacrificed by ear vein injection (Euthanasia-6 Solution) at 21 days. Because severe ocular irritancy resulted from a dose of 0.1 ml, an additional 4 rabbits (2 males, 2 females) were dosed with 0.01 ml for comparison. These rabbits were dosed as described above except the dose was applied directly onto the cornea. Eye examinations were made at 1,24,48 and 72 hours and at 7,10 and 14 days. All 4 animals were sacrificed at 14 days (ear vein injection using Euthanasia-6 Solution).

Year: 1993
 GLP: Yes
 Test substance: Isobutanol (99.9% purity by capillary GC, GC/MS and NMR used to confirm identity).
 Remark: A volume of 0.1 ml of test substance instilled into rabbit eyes produced minor to moderate corneal injury in 2 of 2 rabbits. Iritis and severe conjunctival irritation were also apparent in both rabbits. One rabbit developed severe conjunctival swelling within 1 hour. At 24 hours, both rabbits had hemorrhages of the nictitating membrane. One animal also had a purulent ocular discharge. Within 7 days, corneal vascularization developed on 1 rabbit. Both animals had alopecia of the periocular area (with a small scab on 1) by 9 days. Except for alopecia, 1 rabbit had a normal ocular appearance at 9 through 15 days. Minor conjunctival redness was again evident in this animal at 21 days. Minor conjunctival redness persisted in the other rabbit at this time.) Following the application of 0.01 ml of isobutanol onto 4 rabbit eyes, minor corneal injury was observed in 2. Iritis and moderate to severe conjunctival irritation were apparent in all 4 rabbit eyes. At 48 and 72 hours, 2 rabbits had hemorrhages of the nictitating membrane and/or sclera. One rabbit had a normal ocular appearance at 72 hours and another 2 rabbit eyes were healed at 7 days. All 4 rabbits had a normal ocular appearance by 14 days.
 Reliability: Score = 1, GLP guideline study

DATE: SEPTEMBER 2004

Reference: S.M. Christopher. 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.

5.3 SKIN SENSITISATION

No data available.

5.4 REPEATED DOSE TOXICITY

(a) Preferred value

Species: rat
Strain: Sprague-Dawley (SD)
Sex: male and female
Route of Admin: inhalation
Exposure Period: 13 weeks
Freq. of Treatment: 6 h/day, 5 days/week, 70-73 exposure days (102 day study period)
Post Exposure
Observation Period: None
Doses: 0, 250, 1000, and 2500 ppm
Control Group: yes
NOAEL: 1000 ppm
LOAEL: 2500 ppm
Method: The study consisted of male and female *ad libitum*-fed Sprague-Dawley (SD) rats designated for functional observational battery, motor activity, and neuropathology endpoints (functional observational battery, motor activity, neuropathology; FOB/MA/NP). Groups consisted of 20, 10, 10, and 20 rats/sex for the 0, 250, 1000, and 2500 ppm groups, respectively. Clinical observations, body weights, and feed consumption were recorded weekly. Ophthalmic examinations were conducted on all rats prior to study start and during the 14th week for the 0 and 2500 ppm group. Neurobehavioral tests (FOB, MA) were performed on 15 rats from the 0 and 2500 ppm groups and 10 rats from the 250 and 1000 ppm groups prior to initiation of exposure and during the 4th, 8th, and 13th week of exposure. Animals were killed one week after the last neurobehavioral exam. Five animals per sex were perfused and selected central and peripheral nervous system tissues were retained for histological examination. Blood was collected from a separate group of five rats per sex per group for haematological and serum chemistry determinations. These same five animals received a full necropsy and tissues were weighed and retained for histological exam. The remaining 10 male animals from the control and 2500 ppm group received a gross necropsy. Testes and epididymides from all male animals were weighed. One testis from each male rat that was perfused was processed for histological examination. For the remaining male animals, one testes was immersion fixed and the contralateral testis and epididymides were frozen and homogenisation-resistant sperm(atid) head counts were determined. All retained nervous system tissue from five control and five 2500 ppm rats/sex were examined by light microscopy. All retained tissues from animals designated for tissue and blood collection (5/sex/group) were examined by light microscopy. One testis from 10 male animals from each exposure group were examined by light microscopy.

Year: 1996
GLP: yes
Test substance: isobutanol (>99.9% pure).

DATE: SEPTEMBER 2004

Remark:	A SCOB testing paradigm involving both a fixed-interval (FI) and fixed-ratio (FR) schedules was also included in a separate study that was conducted concurrently.
Results:	NOEL Neurotoxicity = 2500 ppm. There were no morphological or behavioural effects indicative of a persistent or progressive effect of isobutanol on the nervous system at exposure concentrations of up to 2500 ppm. A slight reduction in responsiveness to external stimuli occurred in all treated groups during exposure. However, there was no difference from the control animals with respect to responsiveness during nonexposure periods. No effects were noted during the FOB examinations. Therefore, the slight decrease in responsiveness are likely transient effects from acute exposure to isobutanol. There was a slight (but statistically significant) increase in red blood cell counts, hematocrit, and hemoglobin parameters in the 2500 ppm female rats when compared to the control female rats. Although these effects were considered related to treatment and considered for the derivation of the NOAEL, they were of questionable biological significance due to the slight nature of the effects. There were no changes in ophthalmology, serum chemistry, organ weights, or gross or microscopic pathology that were attributed to isobutanol exposure.
Reliability:	Score = 1, GLP guideline study
Reference:	Branch, D.K., T.A. Kaempfe, D.C. Thake, A.A. Li. 1996. Three Month Neurotoxicity Study of Isobutanol Administered by Whole-Body Inhalation to CD® Rats. Lab. Proj. No. EHL 94075, MSL 14525. Monsanto Company, Environmental Health Laboratory, 645 S. Newstead, St. Louis, MO 63110 for the Oxo-Process Panel, Chemical Manufacturers Association. Also reported in Li, A.A., Thake, D.C., Kaempfe, T.A., Branch, D.K., O'Donnell, P., Speck, F.L., Tyler, T.R., Faber, W.D., Jasti, S.L., Ouellette, R., and M.I. Banton. 1999. Neurotoxicity Evaluation of Rats After Subchronic Inhalation Exposure to Isobutanol. <i>Neurotoxicology</i> 20(6): 889-900.
(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
NOEL:	316 mg/kg
LOEL:	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.
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, standard method with restrictions
Reference:	"Rat Oral Subchronic Toxicity Study Final Report. Compound: Isobutyl Alcohol." Toxicity Research Laboratories, Ltd. Muskegon, MI. TRL Study #032-002 dated 1987.
(c) Species:	Rat
Strain:	Wistar
Sex:	male and female
Route of Admin:	drinking water
Exposure Period:	90 days
Freq. of Treatment:	continuous
Post Exposure	
Observation Period:	N/A
Doses:	0, 1000, 4000, or 16,000 ppm (approx. 80, 340, or 1450 mg/kg bw/day)
Control Group:	yes
NOAEL:	16,000 ppm (approx. 1450 mg/kg)
LOEL:	N/A
Method:	OECD method 408 was followed during this study. Four groups of male and female rats (10/sex/group) consumed water containing isobutanol for 90 days. At the start of the study, the male and female rats had mean weights of 172 and 147 grams, respectively. The drinking water solutions were checked for homogeneity and concentration verification by gas chromatography. Individual body weights and feed and water consumption were collected. Drinking water solutions were prepared fresh twice a week. Ophthalmic exams were conducted prior to study start and at the end of the study. Hematology and clinical chemistry exams were conducted on study day 87, prior to termination of the animals. At the end of the 90-day exposure period, a gross necropsy was performed and liver, kidney, adrenals, and testes were weighed. Tissues required by Guideline 408 were preserved in 4% formaldehyde. Histopathological processing and examination of selected organs was done according to the guidelines. ANOVA and Dunnett's test were used for statistical comparisons.
Year:	1997
GLP:	yes
Test substance:	isobutanol (purity 99.8% by gas chromatography)

DATE: SEPTEMBER 2004

Remark:	Range finding studies conducted prior to this experiment determined that 16,000 ppm isobutanol in drinking water was the maximum amount without palatability problems.
Results:	There were no treatment-related effects on feed or water consumption, body weights, rate of weight gain, or clinical signs noted in the animals consuming water containing isobutanol. The mean daily intake of isobutanol was 0, 75, 300, 1251 mg/kg for the male rats and 0, 91, 385, 1657 mg/kg for the female rats consuming 0, 1000, 4000, or 16,000 ppm isobutanol in the drinking water. There were no treatment-related changes to the eyes upon ophthalmic exam. One animal from the control group was found dead on study day 42. No changes related to isobutanol exposure were noted upon examination of either the hematology or clinical chemistry data. No treatment-related findings were noted upon gross necropsy of the isobutanol-exposed animals. There were no differences in organ weights between the treated and control groups. Upon histopathological examination, both the treated and control groups had sporadic changes in the testes (tubular degeneration and diffuse hyperplasia of Leydig cells), the spleen (minimal increase in extramedullary hematopoiesis), and the kidney (dilatation of the renal pelvis). The incidental and sporadic occurrence of these lesions in both the control and treated groups led the authors to conclude they were unrelated to isobutanol exposure. The 16,000 ppm exposure concentration was considered to be the NOAEL (approximately 1450 mg/kg bw/day) for this study.
Reliability:	Score = 2, standard method with restrictions
Reference:	Schilling, K., Kayser, M., Deckardt, K., Kuttler, K., and Klimisch, H-J. (1997) "Subchronic toxicity studies of 3-methyl-1-butanol and 2-methyl-1-propanol in rats." Human and Experimental Toxicology, 16:722-726.
(d)	
Species:	Rat
Strain:	Sprague-Dawley
Sex:	N/A
Route of Admin:	inhalation
Exposure Period:	3 or 5 days
Freq. of Treatment:	
Post Exposure	
Observation Period:	N/A
Doses:	500 ppm (1.5 mg/L) or 2000ppm (6 mg/L)
Control Group:	
NOAEL:	
LOEL:	
Method:	Only the influence of isobutanol on the cytochrome P450 enzyme system was investigated.
Year:	1985
GLP:	
Test substance:	isobutanol
Remark:	
Results:	Inhalation exposure had no influence on the cytochrome P450 content in liver, kidney, and lungs. In an ex vivo in vitro assay, hepatic microsomal metabolism of n-hexane to 2- and 3-hexanol was reduced by 24 and 30%, respectively, following 3-day exposure to 2000 ppm isobutanol.
Reliability:	Score = 4, original reference not available
Reference:	Aarstad K. et al.: Arch. Toxicol., Suppl.8, 418-421, (1985) as cited in IUCLID.

(e)	Species:	Rat
	Strain:	
	Sex:	N/A
	Route of Admin:	inhalation
	Exposure Period:	4 months
	Freq. of Treatment:	continuous
	Post Exposure	
	Observation Period:	N/A
	Doses:	0.1, 0.5, 3.0 mg/m ³ (0.0001, 0.0005, 0.003 mg/L)
	Control Group:	
	NOAEL:	
	LOEL:	
	Method:	
	Year:	
	GLP:	
	Test substance:	isobutanol
	Remark:	Data taken from English abstract only as part of the original Russian publication.
	Results:	At 0.0001 mg/L – no signs of toxicity. At 0.0005 and 0.003 mg/L, reduction of erythrocyte number, hemoglobin content, cholinesterase and catalase activity were found. Exposure to 0.003 mg/L increased stimulus threshold to trigger the avoidance response to electrostimulation; increased activity of alanine aminotransferase and aspartate aminotransferase was observed.
	Reliability:	Score = 4, original reference not available
	Reference:	Tsulaya, V.R. et al.: Gig. Sanit., 5, 6-9, (1978) as cited in IUCLID.
(f)	Species:	Rat
	Strain:	Wistar
	Sex:	male
	Route of Admin:	drinking water
	Exposure Period:	4 months
	Freq. of Treatment:	continuous
	Post Exposure	
	Observation Period:	N/A
	Doses:	1 M (ca. 74.12 g/L)
	Control Group:	Yes
	NOAEL:	
	LOEL:	
	Method:	
	Year:	1974
	GLP:	no
	Test substance:	isobutanol
	Remark:	
	Results:	According to the report, the average daily intake of isobutanol increased from 6.5 nmol/100 g (average for initial 15 days) to 12.6 nmol/100 g (average for final 15 days). These amounts appear extremely low (range ca. 4.8 – 9.3 mg/kg bw/d). Presumably a misprint occurred in the publication, and the true values are in [umol/100g]. Alternatively, if one assumes a fluid intake of 20 ml/day and a body weight of 250-400 g, the estimated daily intake of isobutanol would amount from ca. 3.7 to 5.9 g/kg. On dissection, the

DATE: SEPTEMBER 2004

		stomachs were enlarged, filled with gas and/or food, and some animals had signs of small-intestinal bleeding and constipation. Changes in liver (fatty, cirrhotic, or fibrotic lesions) were not observed.
	Reliability:	Score = 4, original reference not available
	Reference:	Hillbom M.E. et al.: Res. Comm. Chem. Pathol. Pharmacol., 9,177-180, (1974) as cited in IUCLID.
(g)	Species:	Rat
	Strain:	Wistar
	Sex:	male and female
	Route of Admin:	drinking water
	Exposure Period:	2 months
	Freq. of Treatment:	daily
	Post Exposure	
	Observation Period:	N/A
	Doses:	2 M (ca. 148.24 g/L)
	Control Group:	not specified
	NOAEL:	
	LOEL:	
	Method:	
	Year:	1974
	GLP:	yes
	Test substance:	isobutanol
	Remark:	Data was only available as an abstract and contained insufficient information upon which to evaluate the adequacy of the experimental design, etc.
	Results:	Rats were given 2M solution of isobutanol for 2 months as a sole drinking fluid; this is equivalent to ca. 9.9 g/kg bw/d (dose estimation is based on assumption of a daily fluid intake of 20 ml and 300 g as an average body weight). Histological examination of the liver showed that the content of fat, glycogen, and RNA as well as the size of the liver cells was reduced.
	Reliability:	Score = 4, original reference not available
	Reference:	Hillbom M.E. et al.: Japan J. Stud. Alcohol., 9, 101-108, (1974); cited in WHO: Environmental Health Criteria 65, pp. 93 ff., (1987), as cited in IUCLID.
(h)	Species:	Rat
	Strain:	
	Sex:	
	Route of Admin:	oral unspecified
	Exposure Period:	4 weeks
	Freq. of Treatment:	6 days/week
	Post Exposure	
	Observation Period:	N/A
	Doses:	1/10 and 1/5 of the LD50 (= ca. 310 and 620 mg/kg bw/d, respectively)
	Control Group:	not specified
	NOAEL:	
	LOEL:	
	Method:	
	Year:	1983
	GLP:	
	Test substance:	isobutanol

Remark:	no further details.
Results:	no deaths
Reliability:	Score = 4, original reference not available
Reference:	Kushneva V.S. et al.: Gig. Tr. Prof. Zabol., 1, 46-47, (1983); cited in WHO: Environmental Health Criteria 65, WHO, pp. 93 ff., (1987) as cited in IUCLID.
(i) Species:	Mouse
Strain:	
Sex:	
Route of Admin:	inhalation
Exposure Period:	9 hr/treatment; total exposure period: 52, 79, 135, 177, 223 hr.
Freq. of Treatment:	6 – 25 times
Post Exposure	
Observation Period:	N/A
Doses:	ca. 0.1 – 0.12 ml vapor in 5 liters of air
Control Group:	not specified
NOAEL:	
LOEL:	
Method:	
Year:	1928
GLP:	
Test substance:	isobutanol
Remark:	This study does not meet current standards.
Results:	No narcosis induced; histological findings – fatty benign degeneration of liver and renal parenchyma.
Reliability:	Score = 4, original reference not available
Reference:	Weese H.: Arch. Exp. Pathol. Pharmacol., 135, 118-130, (1928) as cited in IUCLID.
(j) Species:	Mouse
Strain:	
Sex:	
Route of Admin:	inhalation
Exposure Period:	14, 99, or 136 total exposure hours (exposure at times until narcosis)
Freq. of Treatment:	3, 22, 30 narcoses
Post Exposure	
Observation Period:	N/A
Doses:	ca. 0.12 ml vapor in 5 liters of air
Control Group:	not specified
NOAEL:	
LOEL:	
Method:	
Year:	
GLP:	
Test substance:	isobutanol
Remark:	This study is not consistent with current methodology.
Results:	Histological findings – fatty benign degeneration of liver, kidneys, and heart.
Reliability:	Score = 4, original reference not available

DATE: SEPTEMBER 2004

Reference:	Weese H.: Arch. Exp. Pathol. Pharmacol., 135, 118-130, (1928) as cited in IUCLID.
(k) Species:	Rabbit
Strain:	New Zealand white
Sex:	
Route of Admin:	dermal
Exposure Period:	no data
Freq. of Treatment:	4-6 x 24 hr.
Post Exposure	
Observation Period:	72 hr.
Doses:	0.3 ml undiluted test compound
Control Group:	not specified
NOAEL:	
LOEL:	
Method:	
Year:	1986
GLP:	
Test substance:	isobutanol
Remark:	4 to 6 times repeated 24-hr occlusive exposure to 0.3 ml isobutanol caused severe edema and erythema with eschar formation, which persisted throughout the 72-hr post-observation period.
Results:	Isobutanol was judged to be "highly irritant"
Reliability:	Score = 4, original reference not available
Reference:	TSCATS: OTS 0510692, "Seven Day Skin Irritation Study in Rabbits", unpublished report (HAEL No. 86-0129 ACC. No. 900303), Eastman Kodak Co., (1986); cited in BG Chemie (ed.): 2-Methylpropanol-1, in: Toxikologische Bewertung Nr.96, Ausgabe 01/97, BG Chemie, Heidelberg, pp. 3-40, (1997) as cited in IUCLID.

5.5 GENETIC TOXICITY IN VITRO

A. Bacterial In Vitro Test

(a) Preferred value	
Type:	Ames test
System of Testing:	Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537
Concentration:	10-10000 µg/plate
Metabolic Activation:	with and without
Result:	negative
Method:	A pre-incubation assay method was used. The test chemical (0.05 ml) was mixed with Salmonella culture (0.10 ml) and S-9 mix or buffer (0.50 ml) and incubated at 37 °C for 20 minutes. The tubes were capped to prevent release of volatile chemicals. Top agar was added, the tubes were mixed and then the contents plated onto petri plates containing Vogel-Bonner media. Following two days of incubation at 37 °C, the His+ colonies were counted.
Year:	1988
GLP:	no data
Test substance:	isobutanol
Purity:	not provided although obtained from source known to provide high purity.

- Remark:
Concentration: 10000 µg/plate
Reliability: Score = 1, meets national standard methods
Reference: Zeiger, E., Anderson B., S. Haworth, T. Lawlor, and K. Mortelmans. 1988. Salmonella Mutagenicity Tests: IV. Results From the Testing of 300 Chemicals. Environ. Mol. Mutag. 11 (Suppl. 12):1-158.
- (b) Type: Ames test
System of Testing: Salmonella typhimurium TA 1535
Concentration: up to 1 mg/plate (1 mg/3 ml agar)
Metabolic Activation: without
Result: negative
Method other: *S. typhimurium* strain TA 1535 was tested in a standard Ames assay. Briefly, 0.1 ml of the bacterial culture (containing 2×10^8 bacteria), and 0.9 ml of media. The final 1.0 ml contained 100 mM sodium phosphate buffer (pH 7.4), 8 mM MgCl₂, 33 mM KCl. This was mixed with 2.0 ml of top agar (0.6% agar in 0.5% NaCl) and added to a petri dish containing 20 ml of hard agar (1.5% agar in water). The plates were incubated for 48 hours in the dark at 37° C. Mutant colonies were counted with a Biotran III counter. Three plates were used for each exposure concentration, including blanks and positive controls.
Year: 1993
GLP: no data
Test substance: isobutanol
Purity: >99%
Remark: isobutanol was tested as a possible metabolite of isobutyl nitrite, including reaction of isobutyl nitrite with phosphate. Only one concentration was tested in one strain of bacteria.
Concentration: 1 mg/Plate; 1 mg/3 ml agar
Reliability: Score = 2, accepted scientific methods with restrictions
Reference: Mirvish, S.S., Williamson, J., Babcock, D., and Chen, S-C. (1993) Mutagenicity of Iso-butyl nitrite vapor in the Ames test and some relevant chemical properties, including the reaction of iso-butyl nitrite with phosphate. Env. Molecular Mutagen. 21:247-252.
- (c) Type: bacterial gene mutation assay
System of Testing: Escherichia coli CA 274
Concentration: 3-5%
Metabolic Activation: without
Result:
Method other:
Year: 1969
GLP: no data
Test substance: isobutanol
Purity:
Remark: According to WHO, the study is in itself inadequate to assess the mutagenic potential. With concentrations of 3-5% isobutanol, various E. coli strains were killed to a large extent within ca. 35 minutes at 37 degr. Celcius. Upon exposure to 2.5% isobutanol, the number of E. coli CA 274 revertants was increased over controls (ca. 39.7 vs. 5.4 per 10⁹ cells), due to true back mutations. It is questionable whether the excision repair was inactivated in E. coli strain AB 1157 after exposure to 3% isobutanol. The survival rate for E. coli CA 274 exposed to 2.5% isobutanol was estimated to be ca. 1-10%. No further details available.

DATE: SEPTEMBER 2004

Reliability:	Score = 4, original reference not available
Reference:	Hilscher H. et al.: Acta Biol. Med. Germ., 23, 843-852, (1969) as cited in IUCLID.
(d) Type:	Ames test
System of Testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538; Escherichia coli WP2 uvrA
Concentration:	5, 10, 50, 100, 500, 1000, 5000 ug/plate
Metabolic Activation:	with and without
Result:	negative
Method other:	according to B Ames et al. (1975) Mutat Res 31:347-364
Year:	1985
GLP:	no data
Test substance:	isobutanol
Remark:	Preincubation test; 2 plates/concentration. S9 mix was prepared from livers of male SD-rats that were pre-treated with KC500 (polychlorinated biphenyl) at a dose of 500 mg/kg bw 5 days before sacrifice.
Reliability:	Score = 2, standard methods with acceptable restrictions
Reference:	Shimizu H et al. 1985. Jpn J Ind Health 27: 400-419 as cited in IUCLID.
(e) Type:	Ames test
System of Testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration:	10, 33.3, 100, 333, 1000, 3330, 5000 ug/plate
Metabolic Activation:	with and without
Result:	negative
Method other:	according to Maron, D.M. and Ames, B. (1983) Mutat Res 113:173-215
Year:	1992
GLP:	Yes
Test substance:	isobutanol
Remark:	Plate incorporation assay with and without S9 metabolic activation system. Bacteriotoxicity was not observed at the concentrations tested.
Reliability:	Score = 1, GLP guideline study
Reference:	Hazleton Washington: "Mutagenicity Test on CT-516-92 in the Salmonella/Mammalian-Microsome-Mutation Assay (Ames Test)", final report (HWA Study No.: 15318-0-401), submitted to American Cyanamid Co., 12.08.1992; cited in BG Chemie (ed.):2-Methylpropanol-1, in: Toxikologische Bewertung, Ausgabe 01/97, BG Chemie, Heidelberg, pp. 3-40, (1997) as cited in IUCLID.
(f) Type:	Ames test
System of Testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration:	0.0008, 0.008, 0.08, 0.802, 4.01 ug/plate
Metabolic Activation:	with and without
Result:	negative
Method other:	according to Ames, B.N., et al., Mutat Res 31:347-364.
Year:	1975
GLP:	no data
Test substance:	isobutanol
Remark:	Plate incorporation assay with and without S9 mix prepared from liver of Arochlor 1254-induced male SD rats.
Reliability:	Score = 4, original reference not available
Reference:	TSCATS: OTS 0513188, Doc. I.D.: 86-870000238, 02.01.1978, Celanese Chemical Co., Inc.; cited in BG Chemie (ed.): 2-Methylpropanol-1, in:

Toxikologische Bewertung, Ausgabe 01/97, BG Chemie, Heidelberg, pp. 3-40, (1997) as cited in IUCLID.

B. Non-Bacterial In Vitro Test

- (a) Preferred value
 Type: Comet, micronucleus, and HPRT-gene mutation assay
 System of Testing: Comet assay – human lung carcinoma epithelial A549 cells, V79 Chinese hamster fibroblasts, and human peripheral blood cells. Micronucleus assay - V79 Chinese hamster fibroblasts, HPRT-gene mutation assay - V79 Chinese hamster fibroblasts.
- Concentration: up to 270 mM
 Metabolic Activation: Comet assay – none; micronucleus and HPRT-gene mutation assay – with and without S-9 fraction.
- Result: Isobutanol caused cytotoxicity in A549 cells with an IC50 of 11 mM. Isobutanol did not cause genotoxicity in the A549, V79, or human peripheral blood cells.
- Method other: The A549 cell culture was maintained by standard cell culture techniques. Cells in the exponential growth phase were harvested and used in the cytotoxicity (colony forming ability assay) and comet assays. For the cytotoxicity test, cells were grown in media containing the test chemical for 9 days, followed by colony growth determination. A peripheral blood sample was collected from a 59-year old man by venipuncture. For the comet assay, whole blood was incubated with the test chemical for 4 hours at 37° C. At the end of the incubation period, the cells were washed and isolated for use in the alkaline comet assay. A549 and V79 cells were also treated with the test chemical for four hours at 37° C. The cells were then washed and isolated for the alkaline comet assay. The alkaline comet assay was conducted by standard techniques (i.e. cell lysis, alkali treatment for 1 hour, followed by electrophoresis). DNA migration and damage was analysed by fluorescence microscopy and image analysis. The tail moment was calculated. V79 cells were used in the micronucleus assay and were incubated with the test chemical for 4 hours with and without S-9 fraction. After treatment, the cells were washed and incubated for 24 hours, followed by micronuclei counting. Micronucleus frequency was determined. V79 cells were also used for the HPRT assay. The V79 cells were exposed to the test chemical for 2 hours with and without S-9 fraction. Survival and HPRT gene expression frequencies were determined. All experiments were performed in triplicate and mean values were analysed by Student's-T-test with a 3-fold increase in frequency being required prior to being considered positive as a clastogen or a mutagen.
- Year: 2002
 GLP: no data
 Test substance: isobutanol
 Purity: Highest commercially available (typically >99%)
 Remark: Isobutanol was being evaluated as a “microbial volatile organic compound” from fungi metabolism and for occupational indoor air quality issues in composting facilities.
- Reliability: Score=2, valid with restrictions

DATE: SEPTEMBER 2004

- Reference: Kreja, L. and H.-J. Seidel (2002) "Evaluation of the genotoxic potential of some microbial volatile organic compounds (MVOC) with the comet assay, the micronucleus assay, and the HPRT-gene mutation assay." Mutation Research Vol. 513, pp.143-150.
- (b) Type: gene mutation in *Saccharomyces cerevisiae*
System of Testing: *Saccharomyces cerevisiae* strain D4
- Concentration: 0.0008, 0.008, 0.08, 0.802, 4.01 ug/plate
Metabolic Activation: with and without
- Result: negative
- Method other:
Year: 1975
GLP: no data
Test substance: isobutanol
Remark: Plate incorporation assay with and without addition of S9 mix prepared from liver of Arochlor 1254-induced male SD rats.
- Reliability: Score = 4, original reference not available
Reference: TSCATS: OTS 0513188, Doc. I.D.: 86-870000238, 02.01.1978, Celanese Chemical Co., Inc.; cited in BG Chemie (ed.): 2-Methylpropanol-1, in: Toxikologische Bewertung, Ausgabe 01/97, BG Chemie, Heidelberg, pp. 3-40, (1997) as cited in IUCLID.
- (c) Type: mouse lymphoma assay
System of Testing: L5178 cells, TK locus
- Concentration: up to 10 mg/ml without activation; up to 5 mg/ml with activation.
Metabolic Activation: with and without
- Result: negative
- Method other:
Year:
GLP: no data
Test substance: isobutanol
Remark:
- Reliability: Score = 4, original reference not available
Reference: Litton Bionetics: "Mutagenicity Evaluation of Isobutyl Alcohol in the Mouse Lymphoma Forward Mutation Assay", final report (LBI Project No. 20989) to Celanese Chemical Corp., November, (1978); cited in TSCATS: OTS 0532868, Doc.I.D.: 40-91114031, 09.19.1991, OXO Panel CMA, (1991) as cited in IUCLID.

5.6 GENETIC TOXICITY IN VIVO

- (a) Preferred value
- Test substance: isobutanol
Test species/strain: 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

DATE: SEPTEMBER 2004

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 Index or 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, GLP guideline study
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.
(b) Test substance:	isobutanol
Test species/strain:	rat
Test method:	cytogenetic assay by gavage (single treatment) at 1/5 of the LD50. 48 hours after gavage, cells from the bone marrow were studied for cytogenetic effects.
GLP:	no data
Test results:	Treatment resulted in an increased rate of polyploid cells (1.0 +/-0.4% vs. 0.5+/10.3%), cells with chromosomal gaps (0.4+/-0.2% vs. 0.3+/-0.2%) and cells with chromosomal aberrations (1.2 +/-0.5% vs. 0). No further details are available. According to this available data, the result is considered to be negative.
Genotoxic effects:	negative
Comments:	
Reliability:	Score = 4, original reference not available
Reference:	Barilylak I.R. and Kozachuk S.Y.: Tsitol. Genet., 22, 49-52,(1988); cited in BG Chemie (ed.): 2-Methylpropanol-1, in: Toxikologische Bewertung, Ausgabe 01/97, BG Chemie, Heidelberg, pp. 3-40, (1997) as cited in IUCLID.

5.7 CARCINOGENICITY

No data available

5.8 TOXICITY TO REPRODUCTION

(a) Preferred value	
Type:	Two generation study
Species/strain:	Rat/Sprague-Dawley
Sex:	Male and Female
Route of Adm.:	inhalation
Year:	2003
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

DATE: SEPTEMBER 2004

	<p>mating. Exposures continued in the male animals until sacrifice. The female animals were exposed thru gestation day 20, with exposure reinitiated on lactation day 5 and continued thru 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 thru gestation day 20, with exposure reinitiated on lactation day 5 and continued thru lactation day 21. Body weight, feed consumption, exposure parameters, necropsy endpoints, and reproductive and developmental endpoints were collected according to the test guideline.</p>
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
NOEL Parental:	2500 ppm
NOEL F1 Offspring:	2500 ppm
NOEL 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 (Li, 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, GLP Guideline study
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 Panel, 1300 Wilson Boulevard, Arlington, VA 22209.
(b) Type:	
Species/strain:	Rat/Sprague-Dawley
Sex:	Male and Female
Route of Adm.:	inhalation
Method:	The description of the test method is the same as study (a) in the repeated dose toxicity section. Testes from the rats were collected at necropsy and one testis from 10 male animals from each exposure group were examined by light microscopy. The contralateral testis and epididymides were frozen and homogenisation-resistant sperm(atid) head counts were determined. The microscopic examination of the one testis attempted to determine the frequency of testicular stages I-XIV. In the process of shipping the testis to the lab for histological processing, the testes were placed in plastic bags with fixative. The plastic bags were compressed in the shipping container and flattened the tissue, distorting the three-dimensional architecture of the

DATE: SEPTEMBER 2004

	testes. The pathologist tried to conduct the testicular staging exercise with the flattened testes anyway.
Exposure period:	6 hours/day
Freq. of treatment:	7 days/week for 90 days
Exposure conc.:	0, 250, 1000 and 2500 ppm
Control group:	Concurrent
NOEL Parental:	2500 ppm
Results:	Epididymal homogenisation-resistant spermatid head counts were comparable between the treated and control groups. Testicular homogenisation-resistant spermatid head counts were comparable between the control group and the 250 and 2500 ppm groups while the 1000 ppm group was increased compared to the control values. The lack of a dose-response relationship indicated that the increase observed in the 1000 ppm group was unrelated to isobutanol exposure. Frequencies of stages I-XIV were unaffected in all exposure groups other than stage XIII (increased in 2500 ppm group only) and stage XIV (increased in the 1000 ppm group only).
GLP:	yes
Test substance:	isobutanol (>99.9% purity)
Remarks:	The attempt to conduct the stage frequency exercise despite the distorted testes tissue was a unfortunate decision, since the three-dimensional architecture is essential for determining the stage of the testes on cross-section. The lack of histological findings in the other testes, the lack of dose-dependent effects on spermatid head counts, and the lack of interpretable changes in stage frequency indicate that isobutanol did not affect testicular function in these animals. This reasoning is supported by the data from the two-generation reproductive toxicity described as study (a) in this section.
Reliability:	(score =3, methodological deficiencies)
Reference:	Branch, D.K., T.A. Kaempfe, D.C. Thake, A.A. Li. 1996. Three Month Neurotoxicity Study of Isobutanol Administered by Whole-Body Inhalation to CD© Rats. Lab. Proj. No. EHL 94075, MSL 14525. Monsanto Company, Environmental Health Laboratory, 645 S. Newstead, St. Louis, MO 63110 for the Oxo-Process Panel, Chemical Manufacturers Association. Also reported in Li, A.A., Thake, D.C., Kaempfe, T.A., Branch, D.K., O'Donnell, P., Speck, F.L., Tyler, T.R., Faber, W.D., Jasti, S.L., Ouellette, R., and M.I. Banton. 1999. Neurotoxicity Evaluation of Rats After Subchronic Inhalation Exposure to Isobutanol. Neurotoxicology 20(6): 889-900.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

(a)	Preferred value	
	Species:	rat
	Strain:	Wistar
	Sex:	female
	Route of Admin:	inhalation
	Exposure	
	Period	Day 6 thru 15 of gestation
	Frequency of	
	Treatment:	Daily for 6 hours/day
	Duration of	

DATE: SEPTEMBER 2004

Test	10 treatment days/animal
Exposure Conc.	0, 0.5, 2.5, or 10.0 mg/l
Control Group:	yes, concurrent no treatment
LOEL (Maternal Toxicity)	10.0 mg/l
NOAEL (Teratogenicity)	10.0 mg/l
Method other:	Pregnant rats (25/group) were exposed to isobutanol by whole body inhalation from gestation day 6 thru 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
Reference	BASF (c) Klimisch, H.-J. 1990. Prenatal Toxicity of 2-Methyl-1-Propanol in Rats after Inhalation. Project No. 67R057/88047. BASF Department of Toxicology, BASF Corporation. 6700 Ludwigshafen, West Germany.
(b) Preferred value	
Species:	rabbit
Strain:	Himalayan
Sex:	Female
Route of Admin.:	Inhalation
Exposure Period:	Day 7-19 of gestation
Freq. of Treatment:	6 hours/day
Duration of Test:	Up to Day 29 post-implantation
Exposure Concentrations:	0; 0.5; 2.5; 10 mg/L
Control Group:	Yes
NOAEL Maternal Toxicity:	2.51 mg/L
NOAEL Developmental Toxicity:	10 mg/L
Method:	OECD Guide-line 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 (variations in cardiac septal development) were found for the high-dose group. This is a very common variation in rabbits. The litter incidence in

DATE: SEPTEMBER 2004

Reliability: this study was 13.3%, 7.1%, 0%, and 38.5% in the control, low, mid and high exposure groups, respectively. This finding was not considered to be of biological significance, because with the litter historical control range for this variation was from 0 to 47%. Therefore, the incidence in the high exposure group was found to be within the normal range of biological variation for this strain of rabbit. Substance related effects on the offspring, indicative of embryo-/fetotoxicity or teratogenicity, were not observed. Score = 1, GLP guideline study

Reference: BASF (d), 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 TOXICOKINETICS

A. Specific toxicities

(a) Remark: Immunotoxicity – Lymphocyte Mitogenesis Test
Species: Mouse
Strain: C3H/He and BALB/C
Sex: male
Route of Admin: *in vitro* exposure
Exposure Period: 96 hours
Frequency of Treatment: continuous
Duration of Test: 96 hours
Exposure Concentration: 10^{-9} to 10^{-3} mol/L
Control Group: Solvent control (distilled water or DMSO (max. 0.3%))
Method other: B cells were isolated from the spleen of male C3H/He mice following injection of 10 ml of RPMI-1640 medium into the spleen. T cells were isolated from the spleen of male BAIB/C mice in a similar manner. The cells were flushed out of the spleen using a syringe and the suspended cells were removed from the connective tissue, washed, and counted with a hemacytometer. The cells were dispensed into 96-well microplates at 10^5 cells/well in 200 μ L RPMI-1640 medium containing 5 mM HEPES, 50 μ M 2-mercaptoethanol, 100 IU/ml penicillin, 50 μ g/ml streptomycin, 0.18% NaHCO₃, and 10% fetal calf serum. Mitogenic stimuli for the B and T cells were provided by addition of 100 μ g/ml lipopolysaccharide or 200 μ g/ml concanavalin A, respectively. The test chemical was added to stimulated cells and incubations proceeded for 96 hours at 37° C in a 5% CO₂ atmosphere. At the end of the exposure period, the total amount of DNA in the grown cells was determined by the ethidium bromide fluorescence method. The control cells were treated in the same manner as the treated cells with the exception that no test chemical was added (only vehicle). A cell growth curve was plotted against test chemical concentration and a concentration for 50% growth inhibition (IC50) was determined.

Year: 2001
GLP: no data
Test substance: isobutanol (purity >99%)
Result: Isobutanol showed no inhibition of mitogenic activity in stimulated B and T cells in the concentration range tested.

Reference: Sakazaki, H., Ueno, H., Umetani, K., Utsuni, H., and K. Nakamuro. (2001) "Immunotoxicological evaluation of environmental chemicals utilizing the

mouse lymphocyte mitogenesis test.” Journal of Health Sciences, 47(3), pp. 258-271.

- (b) Remark: Contact Urticaria Test
 Species: Human
 Race: Asian (with demonstrated facial flushing sensitivity to oral ethanol exposure)
 Sex: no data
 Route of Admin: dermal
 Exposure Period: single dose
 Frequency of Treatment: single event
 Duration of Test: 10 minutes
 Exposure Concentration: 75% in water
 Control Group: no data
 Method other: Three Asian subjects who previously had reported severe facial flushing in response to oral ethanol ingestion were studied with a patch test using isobutanol. Ethanol was used as a positive control agent. The skin was rated for the presence or absence of erythema immediately post-exposure.
 Year: 1985
 GLP: no data
 Test substance: isobutanol
 Result: Isobutanol was tested by the patch test method for contact urticaria in Asian subjects with a known sensitivity to ethanol. Dermal isobutanol exposure did not cause erythema in any of the test subjects. Ethanol was positive for erythema in these same test subjects.
 Reference: Wilkin, J.K. and G. Fortner (1985) “Ethnic contact urticaria to alcohol.” Contact Dermatitis: Environmental and Occupational Dermatitis, Vol. 112, pp. 118-120.

B. Toxicodynamics, toxicokinetics

- (a) Preferred value
 Species: human
 Strain: N/A
 Sex: Not available
 Route of Admin: oral
 Exposure Period: Two hours
 Freq. of Treatment: Single
 Duration of Test: Eleven hours
 Exposure Concentration: Not reported. Administered as Isobutanol and Ethanol in water to produce a blood isobutanol level of 4 µmol/L whole blood at end of dosing period.
 Control Group: None (biological samples taken prior to exposure)
 Method: In an effort to understand the elimination kinetics of aliphatic alcohols found in alcoholic beverages, research was conducted with human subjects. Test subjects consumed isobutanol in a ethanol/water vehicle over a two hour time period. Blood and urine samples were collected prior to consumption, at the end of the two-hour consumption period, at one, two, eight (urine only), and nine hours after the end of the exposure period. Similar experiments resulted in an oral dose of approximately 5 mg/kg isobutanol. The blood and urine samples were mixed, treated with β -glucuronidase, deproteinated, and esterified with methanol or ethanol to detect the acid or aldehyde “down-stream” metabolites. Blood concentration-time curves were constructed for isobutanol, isobutyraldehyde, and isobutyric acid. Urine concentration-time curves were constructed for isobutanol, isobutyraldehyde, isobutyric acid, propionaldehyde, propionic acid, and succinic acid. The last three metabolites

(propionaldehyde, propionic acid, and succinic acid) are the known metabolites of isobutyric acid.

Year: 1983
 GLP: no
 Test substances: isobutanol, ethanol
 Purity: not provided
 Result: The blood concentrations of isobutanol, isobutyraldehyde, and isobutyric acid were approximately 4, 4, and 17 µmol/L at the end of the consumption period, clearly demonstrating that isobutyric acid was the major metabolite of isobutanol metabolism. While the addition of ethanol to the test beverage definitely altered the rate of isobutanol metabolism (via a competition for metabolic enzymes), the presence of ethanol did not affect how isobutanol was metabolized. Blood levels of isobutanol decreased over the next two hours while the isobutyraldehyde levels slowly increased in the blood. Isobutyric acid levels also decreased after the end of the consumption period. Urinary concentrations of isobutanol peaked at the one-hour post-exposure time point. Urinary levels of isobutyraldehyde peaked at the eight-hour post-exposure time point. Urinary levels of isobutyric acid peaked at the end of the two-hour exposure period. Urinary levels of propionaldehyde roughly followed those for isobutyraldehyde with peak levels of approximately 8 µmol/L. Urinary levels of propionic acid rose after the exposure period ended with plateau levels between 2 and 8 hours of approximately 60 µmol/L. Urinary levels of succinic acid roughly followed the propionic acid urinary elimination curve with peak levels of approximately 30 µmol/L. A diagram was provided in the paper describing the further metabolism of isobutyric acid, ending with propionic acid. The formation of succinic acid from propionic acid is proposed based on the known intermediate metabolism of propionic acid via the citric acid cycle.

Isobutanol, isobutyraldehyde, and isobutyric acid blood levels found following isobutanol administration.

Sampling Time (hours)	Isobutanol*	Isobutyraldehyde*	Isobutyric Acid*
Beginning of dosing	0	0	0
End of dosing – 2 hours	4	4	17
1 hr. post-dose	2	4	14
2 hr. post-dose	1	5	13
9 hr. post-dose	0	5	10

*mean µmol/L whole blood; These values were taken from graphs provided in the paper.

Urine levels of isobutanol, isobutyraldehyde, and isobutyric acid found following isobutanol administration.

Sampling Time (hours)	Isobutanol*	Isobutyraldehyde*	Isobutyric Acid*
Beginning of dosing	0	0	0
End of dosing – 2 hours	30	4	80
1 hr. post-dose	125	6	70
2 hr. post-dose	100	6	70
8 hr. post-dose	50	7	40
9 hr. post-dose	10	6	30

*mean µmol/L urine; These values were taken from graphs provided in the paper.

- Reliability: (score = 2)
Reference: Rudell, E. von, Bonte, W., Sprung, R., and Kuhnholz, B. (1983) "Zur Pharmakokinetik der holheren aliphatischen Alkohole." Beitr. Gerichtl. Med., Vol. 41, 211-218.
- (b) Preferred value
Species: human (6 subjects)
Strain: N/A
Sex: Two males and four females
Route of Admin: oral
Exposure Period: 30 minutes
Frequency of Treatment: Single
Duration of Test : Four hours
Exposure Concentration: 1875 mg/L Isobutanol & 30% (by vol.) Ethanol in distilled water
Control Group: None (biological samples taken prior to exposure)
Method: In an effort to understand the elimination kinetics of aliphatic alcohols found in alcoholic beverages, research was conducted with human subjects. Test subjects consumed a beverage containing 1875 mg/L isobutanol and 30% ethanol over a 30 minute time period. This exposure resulted in an oral dose of approximately 5 mg/kg isobutanol and 0.80 g/kg ethanol. Blood samples were collected prior to consumption, at 30, 45, 60, 90, 120, 145, 180, 210, and 240 minutes after the start of the exposure. The blood samples were analysed by gas chromatography. Blood concentration-time curves were constructed for isobutanol and ethanol.
- Year: 1990
GLP: no
Test substances: isobutanol, ethanol, propanol, methanol
Purity: not provided
Result: The blood concentrations of isobutanol peaked at 45 minutes after the start of the exposure period. The addition of ethanol to the test beverage altered the rate of isobutanol metabolism (via a competition for metabolic enzymes). Blood levels of isobutanol decreased over the remaining time periods. The T1/2 for isobutanol (in the presence of large amounts of ethanol) was 1.46 hours. Peak serum levels of isobutanol were approximately "6 mg/kg" while the blood ethanol levels were reported as approximately "1%"
- Reliability: Score = 2, valid with restrictions
Reference: Bilzer, N., Schmutte, P., Jens, M., and Penners, B-M. (1990) "Kinetik aliphatischer Alkohole (Methanol, Propanol-1, und Isobutanol) bei Anwesenheit von Athanol im menschlichen Korper". (The kinetics of aliphatic alcohols (methanol, propanol-1, and isobutanol) in presence of ethanol in human body"). Blutalkohol, Vol. 27, No. 6, pp.385-409.
- (c) Species: Human
Strain: N/A
Sex: unknown
Route of Admin: N/A (in vitro)
Exposure Period: 10 minutes
Freq. of Treatment: Single
Duration of Test: 10 minutes
Exposure Conc.: 100 µM
Control Group: compared to 2.5 to 10 mM ethanol

DATE: SEPTEMBER 2004

Method:	The roles of different isozymes of alcohol dehydrogenase (ADH) in the metabolism of aliphatic alcohols were investigated. Human liver ADH isoenzymes were prepared from two healthy tissue donors that succumbed to sudden death. Class I, II, and III ADH isoenzymes were isolated using DEAE-cellulose chromatography with affinity chromatography as the final separation step. The enzymes were assayed at 25°C in 50 mM sodium phosphate buffer at pH 7.4 containing 1.5 mM NAD and the respective alcohols. 50 mM semicarbazide was used to prevent the further reaction of the aldehydes to the corresponding acids. The reaction was initiated by the addition of the isoenzyme and stopped by the addition of ortho-phosphoric acid. The addition of the acid also liberated the respective aldehydes that were then analysed in the vial headspace by gas chromatography. All runs were assayed in triplicate. The reaction time was such that the aldehyde increased linearly with isoenzyme concentration. An additional check was to correlate the concentration of the aldehyde with the increase in NADH concentration (determined spectrophotometrically). Kinetic constants were estimated from the initial rate equations using a simplex algorithm with standard deviations estimated using Monte Carlo sensitivity analysis.
Year:	1988
GLP:	no
Test substance:	isobutanol
Result:	Class I ADH had a Km of 33 µM and a Vmax of 0.19 IU/mg protein for isobutanol. The resulting Class I activity (IU/mg) was 0.14 while the Class II ADH activity was 0.0004. Class III activity was below the limit of detection. These results demonstrate that the Class I ADH activity is primarily responsible for the oxidation of isobutanol in the human liver and that isobutyraldehyde is the product of the reaction.
Reliability:	(score = 2)
Reference:	Ehrig, T., Bohren, K.M., Wermuth, B., and von Wartburg, J-P. (1988) "Degradation of Aliphatic Ethanol and Pharmacokinetic Implications." Alcoholism: Clinical and Experimental Research, Vol. 26, No. 6, pp. 789-794.
 (d)	
Species:	Human, rat, chick embryo
Strain:	Human - N/A, rat – Sprague-Dawley, chick embryo - unknown
Sex:	Human and chick embryo – unknown, rat - female
Route of Admin:	N/A (in vitro)
Exposure Period	40 seconds
Freq. of Treatment:	Single
Duration of Test	40 seconds
Exposure Concentration	100 µM
Control Group:	0.8 to 3 mM ethanol
Method:	The relative metabolic rate constants of aliphatic alcohol metabolism by liver supernatants from several species were investigated. Supernatants (100,000 g) prepared from human hepatocytes from four tissue donors were measured for alcohol dehydrogenase (ADH) activity. The supernatants were prepared in Hepes-DTT-sucrose buffer. The supernatants were diluted in a Tris-phosphate buffer (ph = 7.3) assayed at 38°C after the addition of 3 mM NAD+ and isobutanol. The rates of NADH formation were followed at 340 nm for 40 seconds at each substrate concentration using a spectrophotometer. Semicarbazide was used to prevent the further reaction of the aldehydes to the corresponding acids. All reactions followed the Michealis-Menten kinetics and Vmax and Km was calculated using the Lineweaver-Burke method.
Year:	1990
GLP:	no

DATE: SEPTEMBER 2004

Test substance:	isobutanol
Result:	Rat liver supernatant ADH activity had a Km of 0.05 μM and a Vmax of 1.07 $\mu\text{mol min}^{-1} \text{g wet wt.}^{-1}$. Human liver supernatant ADH activity had a Km of 0.04 – 0.11 μM and a Vmax of 0.68 – 0.86 $\mu\text{mol min}^{-1} \text{g wet wt.}^{-1}$. Chick embryo liver supernatant ADH activity had a Km of 0.22 μM and a Vmax of 0.29 $\mu\text{mol min}^{-1} \text{g wet wt.}^{-1}$.
Reliability:	(score = 2)
Reference:	Sinclair, J., Lambrecht, L., and E.L. Smith (1990) "Hepatic Alcohol Dehydrogenase Activity in Chick Hepatocytes Towards the Major Alcohols Present in Commercial Alcoholic Beverages: Comparison with Activities in Rat and Human Liver." <i>Comp. Biochem. Physiol.</i> Vol. 96B, No. 4, pp.677-682.
 (e)	
Species:	Rat
Strain:	Wistar
Sex:	Male and Female
Route of Admin:	Intraperitoneal, liver perfusion, in vitro liver homogenate
Exposure Period	In vivo – 7 hours, perfusion – 60 minutes, in vitro – 30 minutes
Freq. of Treatment:	Single
Duration of Test	Up to 7 hours
Exposure Concentration	In vivo – 237 mg/kg isobutanol, 1,569 mg/kg ethanol Perfusion – 26.5 mmoles/liter isobutanol and ethanol, in vitro – 11 mM isobutanol, 1100 mM ethanol
Control Group:	In vivo – pre-injection samples, perfusion and in vitro - yes
Method:	The metabolism of isobutanol in rats was investigated. In vivo – two rats received an intraperitoneal injection of isobutanol and ethanol. Blood samples were collected via the tail vein after 15, 45, 75, and 105 minutes and then after every hour for up to 7 hours and analysed for each of the alcohols by gas chromatography. Perfusion – rats were anaesthetized with Nembutal (demonstrated not to interfere with ethanol metabolism) and the hepatic portal vein and the hepatic vein were cannulated. A blood:saline mixture to which isobutanol and ethanol had been added (final concentration of each - 26.5 mmoles/liter) was used to perfuse (2 ml/minute) the liver in situ for 60 minutes. These experiments were repeated with 2mM pyrazole added to the mixture. Samples were collected at 15-minute intervals and analysed by gas chromatography for each of the alcohols. In vitro – A supernatant was produced by homogenizing adult rat livers followed by centrifugation at 800 x g for five minutes. Isobutanol was added (either alone or with ethanol) with 2 mM NAD to initiate the reaction. These experiments were repeated with 2mM pyrazole added to the mixture. The incubation flasks were shaken at 30C for 30 minutes with samples taken for analysis for gas chromatography at 0, 15, and 30 minutes. Pyrazole was added to both the in situ and in vitro experiments to inhibit the metabolism of the alcohols by alcohol dehydrogenase.
Year:	1969
GLP:	no
Test substance:	Isobutanol and ethanol
Result:	Isobutanol reached levels in blood of approximately 0.1 mg/ml at 15 minutes post-injection and these levels decreased only slightly over the 7 hour test period. Blood ethanol levels reached peak levels of 0.7 mg/ml and decreased over 5 hours to baseline. Blood levels of isobutanol did not start to decline appreciably until ethanol blood levels reached 0.2 mg/ml. Pyrazole inhibited the metabolism of both isobutanol and ethanol by the same degree, supporting the idea that both of these alcohols were metabolized by alcohol dehydrogenase. Rat liver perfusion experiments

DATE: SEPTEMBER 2004

results indicated that isobutanol was metabolized at a rate of 0.06 mM isobutanol/gram of liver during the first 30 minutes. Isobutanol was metabolized more rapidly than ethanol or isoamyl alcohol but slower than n-propanol in the perfused rat liver. Liver homogenates from male and female rats metabolized both ethanol and isobutanol at equal rates, demonstrating a lack of gender differences for metabolism. Pyrazole inhibited the metabolism of both ethanol and isobutanol by alcohol dehydrogenase in vitro. Isobutanol was metabolized by the rat liver homogenate in vitro system at a rate of 0.2 mM/g liver in 30 minutes. Similar to the liver perfusion results, isobutanol was metabolized more rapidly than ethanol or isoamyl alcohol but slower than n-propanol in the in vitro system.

Reliability: (score = 2)
Reference: Hedlund, S-G. and Kiessling, K-H. (1969) "The Physiological Mechanism Involved in Hangover 1. The Oxidation of Some Lower Aliphatic Fusel Alcohols and Aldehydes in Rat Liver and Their Effects on the Mitochondrial Oxidation of Various Substrates" Acta Pharmacol. Et Toxicol. Vol.27, pp. 381-396.

(f) Species: Rabbit
Strain: Not available
Sex: Male
Route of Admin: Oral
Exposure Period: Single administration
Freq. of Treatment: Single
Duration of Test: Six hours
Exposure Concentration: 2 ml/kg
Control Group: None
Method: The metabolism of isobutanol in rabbits was investigated. Anaesthetized male rabbits were administered isobutanol and arterial blood samples were taken at 30 minutes, and 1, 2, 3, 4, 5, and 6 hours post-dosing. Blood levels of isobutanol were analysed by gas chromatography. A separate group of animals were evaluated for excretion of isobutanol and metabolites in the urine and exhaled air. Urine was collected via a bladder catheter while exhaled air was collected with a mask and one-way valve. Levels in urine and expired air were measured by gas chromatography. Rabbit liver microsomes were prepared and the ability of this in vitro preparation to metabolise isobutanol was determined. An additional experiment described rabbits dose orally with 2 ml/kg or isobutanol followed by consumption of water containing 20% (v/v) isobutanol. Urine was collected and analysed by gas chromatography.

Year: 1975
GLP: no
Test substance: isobutanol
Result: Isobutanol blood levels peaked at 1 hour post-dosing with blood levels of approximately 0.8 mg/ml. Blood levels decreased over the next 3 hours and were near zero by 4 hours post-dosing. Blood pH levels dropped to 7.2-7.3 from the 30-minute time point until 4 hours post-dosing. Changes in blood pH were considered due to depressed respiratory activity and not due to the production of metabolites (e.g. isobutyric acid). Rabbit liver homogenates metabolized isobutanol at rates approximately equal to ethanol (results of a previous experiment). Very little (0.5%) of the isobutanol administered orally was excreted in the urine or exhaled air. Urinary levels of isobutyraldehyde were 0.12 mg/ml while isobutyric acid was present in trace amounts. Unexplainable levels of isovaleric acid (1.6 mg/ml) were

DATE: SEPTEMBER 2004

found in the urine of the rabbits receiving oral dose of isobutanol and isobutanol in the drinking water. The metabolite described as isovaleric acid may have been another metabolite (described in the paper by Rudell, et al.; see (a)) co-eluting with isovaleric acid on the chromatogram.

- Reliability: (score = 2)
Reference: Saito, M. (1975) "Studies On The Metabolism Of Lower Alcohols" N.U. Med. J. Vol. 34, pp. 569-585.
- (g) Species: Human
Strain: Not available
Sex: Not available
Route of Admin: in vitro
Exposure Period: Single administration
Freq. of Treatment: Single
Duration of Test: Not available
Exposure Concentration: 1 nM
Control Group: Compared to ethanol
Method: The metabolism of isobutanol in human skin samples was investigated. Homogenates of human skin were prepared and alcohol dehydrogenase activities determined for a series of alcohols. Attempts were made to correlate enzyme activity with the frequency of erythemogenesis observed in a test population of human subjects.
- Year: 1987
GLP: no
Test substance: isobutanol
Result: Human skin alcohol dehydrogenase enzymatic activity for isobutanol was 103.7 nM/mg protein-minute. Corresponding values for ethanol were 98.1 nM/mg protein-minute. Two of twelve test subjects had erythemogenic reactions to isobutanol.
- Reliability: score=2
Reference: Wilkin, J.K. and Stewart, J.H. (1987) "Substrate Specificity of Human Cutaneous Alcohol Dehydrogenase and Erythema Provoked by Lower Aliphatic Alcohols" J. Invest. Dermatol. Vol. 88, pp. 452-454.
- (h) Preferred value
Species: rat
Strain: Sprague-Dawley
Sex: male
Route of Admin: inhalation
Exposure Period: Two hours
Freq. of Treatment: Single
Duration of Test: two hours
Exposure Concentration: 2000 ppm (the chamber is charged with 2000 ppm isobutanol and the concentration drops as the rat inhales the test article. Loss to chamber equipment and external surface of the rat is corrected for).
Control Group: None (biological samples taken prior to exposure). The amount inhaled by the rat (versus deposited on chamber equipment surfaces is corrected for).
Method: In an effort to understand the respiratory bioavailability of aliphatic alcohols and esters, a whole-body plethysmograph was installed in a gas-uptake chamber. The rat has an indwelling jugular cannula implanted prior to study start and is placed in the plethysmograph. The plethysmograph (containing the rat) is then placed in the gas-uptake chamber. The leads from the plethysmograph and the venous catheter are exteriorized from the chamber for sample and data collection. The chamber is charged with 2000-ppm

isobutanol and the chamber concentration decay curve is followed by gas chromatography. In addition, venous blood samples are taken at 0, 5, 10, 20, 25, 30, 40, 50, 60, and 90 minutes. The whole-body plethysmograph is designed to measure (non-invasively) ventilatory movements on conscious rats. By collecting data on ventilatory movements, and chamber and venous blood isobutanol concentrations, respiratory bioavailability determinations can be calculated. Blood samples were analyzed for isobutanol (N=7) and isobutyric acid (N=2) concentrations.

Year: 2003
 GLP: no (conducted in spirit of GLP, but not specifically)
 Test substances: isobutanol
 Purity: Spectroscopic grade (>99.9%)
 Result: The blood concentrations of isobutanol and isobutyric acid during the exposure period are reported below. The presence of isobutyric acid following isobutanol inhalation exposure clearly demonstrates that isobutyric acid was the major metabolite of isobutanol metabolism. Blood levels of isobutanol increased up to 277 µM at 15 minutes into the exposure, and declined over the remaining 70 minutes. Chamber concentrations decline from time zero, both due to loss to chamber equipment surfaces as well as uptake by the rat (data not shown). Isobutyric acid levels increased up to 93 µM at 25 minutes, after which they declined to 40 µM at 60 minutes.

Isobutanol and isobutyric acid blood levels found following isobutanol inhalation.

Sampling Time (minutes)	Isobutanol*	Isobutyric Acid*
0	0	0
5	169	8
10	254	18
15	278	43
20	264	55
25	240	93
30	252	91
40	248	42
50	233	39
60	243	40
90	155	ND

*mean µM whole blood (N=7 for isobutanol; N= 2 for isobutyric acid)

Reliability: Score=2, valid with restrictions
 Reference: Poet, T. (2003) Unpublished data. Battelle, Pacific Northwest National Laboratory, US Dept. of Energy. For Oxo-Process Panel, Chemstar, American Chemistry Council, Arlington, VA, 22209.

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

No data available

6.0 REFERENCES

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