FOREWORD INTRODUCTION

ISOPRENE

CAS N°: 78-79-5

SIDS Initial Assessment Report

For

SIAM 20

Paris, France, 19-22 April 2005

1. Chemical Name	Isoprene
2. CAS Number	78-79-5
3. Sponsor Country	United States National SIDS Contact Point in Sponsor Country: Oscar Hernandez, Director U.S. Environmental Protection Agency Risk Assessment Division (7403 M) 1200 Pennsylvania Avenue, NW Washington DC 20460 Phone: (202) 564-7461
4. Shared Partnership	Olefins Panel, American Chemistry Council
5. Roles/Responsibilities of the Partners	
• Name of industry sponsors /consortium	Olefins Panel, American Chemistry Council / Elizabeth Moran ExxonMobil Biomedical Sciences, Inc. / Dan Caldwell
• Process used	Documents drafted by ExxonMobil Biomedical Sciences, Inc. P.O. Box 971, 1545 Route 22 East, Annandale, NJ 08801-0971, USA. Reviewed by Olefins Panel, American Chemistry Council industry toxicologists and by United States Competent Authority.
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme?	Industry sponsored the assessment of isoprene under the ICCA Initiative with agreement by the United States (US) to act as sponsor country.
7. Review Process Prior to the SIAM	Industry prepared documents intended for consideration at SIAM 20 that were peer-reviewed within industry. The industry draft dossier, SIAR, and SIAP were submitted to the US, Environmental Protection Agency (EPA) for review prior to their posting on the CDG. Comments from Competent Authorities on the submitted documents received through the CDG will be reviewed by industry and the EPA, and appropriate changes applied.

8. Quality Check Process	**Industry Consortium:** Critical biological studies discussed in the SIAR were reviewed for quality by industry and assigned a reliability code, based on the review process guidance of Klimisch *et al.* (1997). Robust summaries of critical data were added to a SIDS dossier for isoprene and flagged as "critical", the summary formats for selected endpoints were largely based on descriptions in the OECD Form and Guidance for preparing and submitting the SIDS DOSSIER (INCLUDING ROBUST STUDY SUMMARIES), which is from the Manual for Investigation of HPV Chemicals. **US Government:** US EPA peer-reviewed the SIDS documents and audited selected key studies to check the robust study summaries.
9. Date of Submission	21 January 2005
10. Date of Last Update	29 July 2005
11. Comments	None

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	78-79-5
Chemical Name	1,3-Butadiene, 2-Methyl- (Isoprene)
Structural Formula	C_5H_8 (CH_2=C(CH_3)-CH=CH_2)

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

1,3-Butadiene, 2-methyl- (isoprene) is formed endogenously at the rate of 1.9 umol/kg per hour in both rats and mice and at the rate of 0.15 umol/kg per hour in humans. Isoprene is metabolized by microsomal cytochrome P-450 dependent monooxygenases to two monoepoxide metabolites, i.e., 3,4-epoxy-3-methyl-1-butene (EPOX-1) and 3,4-epoxy-2-methyl-1-butene (EPOX-2). These metabolites may then be hydrolyzed, conjugated with glutathione, or further oxidized to the isoprene diepoxide, i.e., 2-methyl-1,2:3,4-diepoxybutane. The intrinsic rates of formation of monoepoxides in human, rat and mouse liver microsomes are roughly similar, when epoxide hydrolase is inhibited, whereas the amount of monoepoxides at the end of incubation can be two to 15 times higher in mouse liver microsomes than in rat and human liver microsomes, respectively. A physiological toxicokinetic model has been developed for inhaled isoprene in mice, rats and humans, taking into account published or assumed kinetic parameters. On the basis of this model, at human exposure conditions (up to 50 ppm [140 mg/m^3]), rates of metabolism are about 14 times faster in mice and about eight times faster in rats than in humans. As the epoxide metabolites are likely responsible for the toxic effects of isoprene, this may explain why the mouse is more susceptible to isoprene toxicity than the rat.

Isoprene has a low potential for acute toxicity. In rats and mice, the oral LD_{50} of isoprene is in the range of 2,043 to 2,210 mg/kg. The 4-hour rat LC_{50} is 64,620 ppm (180,037 mg/m^3) and the 2-hour mouse LC_{50} is 56,363 ppm (157,033 mg/m^3). In humans, isoprene vapors are irritating to the eyes, nose and throat. Liquid isoprene is irritating to the eyes and skin. Data from a 13-week repeated dose study conducted in mice and rats found degeneration of the olfactory epithelium in male mice only at the highest concentration, i.e., 7,000 ppm (19,503 mg/m^3), but not at lower concentrations. IARC reports that in isoprene rubber production workers, subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract were observed. Prevalence and degree were correlated with increasing length of service.

Repeated dose studies demonstrate clear species differences between rats and mice in susceptibility to isoprene. In a 2-week repeated dose inhalation study, the NOAEL for rats was 7,000 ppm (19,503 mg/m^3), the highest dose tested. However, in this same study, exposure of mice to isoprene produced changes in hematological parameters(decreased hematocrit, hemoglobin, erythrocytes), body and organ weights (increased liver weights, decreased thymus, spleen and testes weights) and also produced microscopic lesions in certain tissues (testes, thymus, liver, nasal cavity, forestomach) at levels as low as 438 ppm (1,220 mg/m^3). Thus, 438 ppm was the LOAEL for mice. Similarly, in the 13-week repeated dose inhalation study, the NOAEL for rats was 7,000 ppm (19,503 mg/m^3). In mice, however, hematological effects indicative of a nonresponsive macrocytic anemia and histopathological changes (forestomach, olfactory epithelium, liver) were observed at exposures of 700 ppm (1,950 mg/m^3) and higher. The NOAEL for mice in the 13- week repeated dose study was 220 ppm (613 mg/m^3). Isoprene was tested for mutagenicity in a series of *in vivo* and *in vitro* studies. Isoprene was not genotoxic in any of the *in vitro* assays conducted. However, when exposed by inhalation, isoprene was clearly genotoxic to mouse bone marrow *in vivo*.

Two-year inhalation carcinogenicity studies were conducted with isoprene in B6C3F1 mice and F344 rats. There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, there were no significant increases in the incidence of malignant tumors. Isoprene exposures in rats were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types makes it difficult to

determine if they are truly exposure related. Based on the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as reasonably anticipated to be a human carcinogen and IARC has classified it as 2B; possibly carcinogenic to humans.

Isoprene did not produce any maternal or developmental toxicity in rats following exposure to concentrations as high as 7000 ppm. However, both maternal and developmental toxicity were evident in mice. In mice, both maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm). Significant reductions in fetal bodyweights were observed at the 280 ppm dose level for female fetuses and at the 1400 ppm level for male fetuses. Thus, in this study, 1400 ppm was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm.

Isoprene did not produce any significant effects on reproductive endpoints in rats. However, significant effects on reproductive endpoints were observed in male mice exposed to isoprene at concentrations of 700 ppm (1,950 mg/m^3) and higher. These effects included testicular atrophy as well as decreases in epididymal weight, sperm head count, sperm concentration, and sperm motility. In female mice exposed to 7,000 ppm (19,503 mg/m^3), the average estrous cycle length was significantly longer than that of the control group. Thus, in this study, 70 ppm (195 mg/m^3) is the NOAEL for reproductive effects in male mice and 700 ppm (1,950 mg/m^3) is the NOAEL for female mice.

Environment

Isoprene is a liquid at 25° C with a reported melting point of –145.9° C, a boiling point of 34.0° C, and vapour pressure of 733.3 hPa (25° C). Isoprene has a water solubility of 642 mg/l (25° C), a log K_{ow} of 2.42, and a density of 0.681 g/cm^3 (25° C).

In the air, isoprene has the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with a calculated degradation half-life of 1.2 hours depending on hydroxyl radical concentration. Aqueous photolysis and hydrolysis will not contribute to the transformation of isoprene in aquatic environments because it is either poorly or not susceptible to these reactions.

Results of Mackay Level I distribution modeling at steady state show that isoprene will partition primarily to the air compartment (99.9%), with a negligible amount partitioning to water (0.06%) and soil (0.02%). Level III modeling predicted greatest distribution (99.96%) to the primary compartment of release; when equal releases were assumed, the predicted distribution was: water (88%), soil (9.0%), air (3.1%), and sediment (0.21%).

Isoprene biodegraded to 61 % after 28 days in an OECD 301F study, but was not readily biodegradable because the replicate data exceeded the allowable range (53 to 75%). In an OECD 301D study, isoprene biodegraded to an extent of 2 and 58% in duplicate samples after 28 days, and showed no inhibitory effect in a supplementary study. The supplementary study resulted in 64% biodegradation on day 7, using the acclimated inoculum from the initial study. These data show that isoprene can exhibit high extents of biodegradation once acclimation has occurred. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate. The measured BCF is reported as 5 to approximately 20. The calculated BCF is 15.

Acute aquatic toxicity values for a fish and invertebrate are 7.4 (96hr-LC_{50}) and 5.8 (48hr-EC_{50}) mg/L, respectively. For algae, the 72- and 96-hr EC_{50} is 15 mg/L for biomass and >35 mg/L for growth rate. The algae 72- and 96-hr NOEC is 1.7 and 6.0 mg/L for biomass and growth rate, respectively.

Exposure

Isoprene is a petrochemical that is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount of isoprene is used to manufacture specialty chemicals, intermediates and derivatives which are then used in the production of vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners. The European Union has evaluated isoprene in the framework of Food Contact Material (CS/PM/3351/21640).

Total world isoprene consumption was reported as over 700,000 metric tons in 2004. Most isoprene production is consumed in the country of origin. Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture. In the United States, isoprene production in 1995

was approximately 619 million lb (281,000 metric tons).

Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used. A WEEL (Workplace Environmental Exposure Limit) of 2 ppm was established by AIHA in 2004. The WEEL was 50 ppm prior to the 2004 revision. Isoprene concentration in 426 workplace air samples (4-hr or greater) taken at 3 major isoprene or isoprene polymer producers in the United States from 1993 to 1998 showed that 81% were below 0.5 ppm, 91% were below 1 ppm, and 99% were below 10 ppm.

There are no direct sales to consumers. However, isoprene is used in production of polymers used in paint resins, tyres, footwear, moduled goods, adhesives, motor oil viscosity improvers. Isoprene monomer residual concentration was not detectable in isoprene-derived polymer samples at an analytical sensitivity of 0.1 ppm in work conducted prior to June 1998. Subsequent work in latter 1998, with an increased analytical sensitivity of 0.02 ppm, that evaluated polyisoprene samples demonstrated that 17 out of 19 samples had no detectable isoprene monomer residual, while 2 samples contained between 0.04 and 0.02 ppm. Consequently, potential for consumer exposure will be negligible.

The greatest potential for exposure to isoprene in the environment is in the air compartment because of its high vapor pressure. Partitioning to air from aquatic and terrestrial compartments would occur rapidly due to isoprene physico-chemical characteristics. As such, isoprene has an overall low potential for exposure in environmental compartments other than air. However, its persistence in air is short lived as a result of degradation processes, which suggests that exposure to isoprene will be limited in the environment.

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

The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (irritation, genotoxic, reproductive and developmental toxicity, carcinogenic) and the environment (fish, invertebrates, algae). Based on data presented by the Sponsor country, relating to production in one country which accounts for approximately 40% of global production and relating to the use pattern in one country, under normal manufacturing, formulation, industrial and consumer use of polymerized isoprene containing products, this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number	78-79-5
IUPAC Name	1,3-Butadiene, 2-Methyl-
Molecular Formula	C_5H_8
Structural Formula	CH3 | CH2=C-CH=CH2
Molecular Weight	68.12
Synonyms	Isoprene; 2-Methyl-1,3-Butadiene; 2-Methylbutadiene; 3-Methyl-1,3-Butadiene, Methylbivinyl; Beta-Methylbivinyl; Hemiterpene

1.2 Purity/Impurities/Additives

Isoprene purity is 99+% w/w. Impurities in isoprene can include isoprene dimer (dipentene) at less than or equal to 0.5 % w/w. Isoprene can contain 150 to 250 ppm p-tert butyl catechol added as a stabilizer.

1.3 Physico-Chemical Properties

Table 1. Summary of Physico-Chemical Properties for Isoprene

Property	Value	Reference/Comment
Physical state (25° C)	Liquid	None
Molecular Weight	68.12 (c)	None
Melting point (°C)	-145.9 (m)	O'Neil et al. (2001)
Boiling point (°C)	34.0 (m)	O'Neil et al. (2001)
Relative density (g/cm^3 at 20° C)	0.681 (m)	Budavari S (1996)
Vapor pressure (hPa at 25° C)	733.3 (m)	Zwolinski and Wilhoit (1971)
Water solubility (mg/l at 25° C)	642 (m)	McAuliffe (1966)
Partition coefficient n-octanol/water (log K_{ow})	2.42 (m)	CITI (1992)
Henry's Law constant (HLC) ($Pa*m^3$/mole at 25° C)	7,781 (c)	HLC was calculated using a water solubility of 642 mg/L, vapor pressure of 733.3 hPa, and molecular weight of 68.12.
Partition coefficient organic carbon/water (log K_{oc})	1.83 (c)	EPIWIN (1999)

(m) measured (c) calculated

2 GENERAL INFORMATION ON EXPOSURE

Exposure to isoprene may occur at workplaces where it is manufactured. Based on physical properties, the primary workplace exposure would be by inhalation. No consumer exposure is foreseen because there are no direct sales to consumers.

2.1 Production Volumes and Use Pattern

Isoprene is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount of isoprene is used to manufacture specialty chemicals, intermediates and derivatives, which are then used in the production of vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners. Coblockpolymers (SIS polymers) are used in the production of paint resins, tires, footwear, moduled goods, adhesives, and motor oil viscosity improvers. Also of commercial importance is the chemical conversion of relatively small amounts of isoprene to terpens which are used extensively in flavours and fragrances (Taalman, 1996).

Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture (SRI International, 2000). In the United States, isoprene production in 1995 was approximately 619 million lb (281,000 Mg [metric tons) (USITC, 1995).

Isoprene is obtained by extractive distillation from an isoprene concentrate stream produced by the ethylene production process. In the pyrolysis furnaces of the ethylene production process, paraffinic feedstocks such as ethane, propane, naphthas or gas oils, are subjected to high temperatures in the presence of steam. These conditions result in the partial conversion or cracking of the hydrocarbon feedstock components and formation of unsaturated hydrocarbons. Ethylene and propylene are the primary products, but other olefins, diolefins, aromatics and cyclics are also produced, including a relatively small amount of isoprene. The ethylene process compresses and separates the pyrolysis furnace effluent into product streams. Isoprene produced in the cracking furnace is contained in one of these product streams, the pyrolysis gasoline.

Pyrolysis gasoline is a complex hydrocarbon mixture, consisting predominately of carbon number five (C5+) and higher hydrocarbon components. Distillation of pyrolysis gasoline produces a pyrolysis C5 stream. This stream is also a complex mixture, and consists predominately of the carbon number 5 olefins and diolefins that were produced in the ethylene process cracking furnaces. The stream also includes n-pentane and iso-pentane, which may result largely due to the unconverted pentanes in the ethylene process feedstock. Further processing of the C5 stream results in an isoprene concentrate stream that is separated from the Pyrolysis C5 by a series of distillation and “heat soak” operations. The “heat soak” is used to convert cyclopentadiene to its dimer (dicyclopentadiene) in order to facilitate isolation of the isoprene concentrate. Isoprene concentrate thus produced from the pyrolysis C5 stream has a typical isoprene content of 40%. This concentrate is then processed in an extractive distillation unit that uses a solvent such as acetonitrile to facilitate isolation of the contained isoprene as a 99% purity product.

Isolation of isoprene from the ethylene process co product streams as described above is the primary source of isoprene. “Only this method of production is practiced in the United States and Western Europe. On-purpose synthetic routes to isoprene are also used commercially, including dehydrogenation of isoamylene and isopentane (capacity in Russia) and reaction of isobutylene with formaldehyde (Russia and Japan)” (Kaelin *et al.*, 2005).

2.2 Environmental Exposure and Fate

Isoprene will partition largely into the atmosphere because it has a relatively high vapor pressure at ambient temperatures. Results from an equilibrium distribution model support that isoprene will partition predominantly to the air compartment. Once in the air, wet deposition of isoprene from the air is not likely to play a significant role in its atmospheric fate due to its short half-life in air. In the air isoprene has the potential to rapidly degrade through an indirect photolytic process mediated by hydroxyl radicals (•OH). In comparison, direct photolysis as well as hydrolysis are not expected to contribute to the removal of isoprene from the environment because it is not subject to these degradative processes. Isoprene has the potential to biodegrade to a significant extent based on results of ready biodegradation testing. However, microbial metabolism may not greatly contribute to its removal from the environment because of its potential to rapidly volatilize from aquatic and terrestrial media. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate.

2.2.1 Sources of Environmental Exposure

Higher plants emit volatile hydrocarbons including isoprene and monoterpenes into the atmosphere. The world wide emission rate of these natural hydrocarbons has been estimated to range from 1.8 to 8.3 E11 kg/yr, which exceeds that of non methane hydrocarbons originating from human sources (Brookhaven National Laboratory, http://www.face.bnl.gov/Modelling/isoprene.htm). Typically, 1-2% of CO_2 fixed in photosynthesis is emitted as isoprene, and globally it is estimated that 2.72 to 3.63 E11 kg of isoprene is emitted by forests each year (Fall, http://www.colorado.edu/Chemistry/directory.dir/faculty.dir/biochem.dir/fall.dir/fallres.html).

Presumably the expected exposure from industrial or anthropogenic sources of isoprene throughout the industrial life-cycle would be less than that from natural sources.

Daily isoprene emission rates positively correlate with leaf temperature, which suggests that there is a correlation with time of day. Emissions of isoprene in large forested regions may be significant to the extent that oxidative/reductive balances are influenced. Plant-emitted isoprene contributes to rural ozone concentrations in summer (Guenther *et al.*, 1991; Mendis, 1994; Monson *et al.*,1994). Isoprene polymers also occur naturally. The natural rubber caoutchouc is *cis* -1,4-polyisoprene, and *trans* -1,4-polyisoprene is present in the natural rubbers balata and gutta-percha. (Columbia Encyclopedia, 2005; http://www.encyclopedia.com/html/i1/isoprene.asp).

Song *et al.* reported on sources of isoprene in Harris County, TX, USA (Table 2) from the Texas Air Quality Study (TexAQS, www.utexas.edu/research/ceer/texaqs) conducted in August and September 2000.

Table 2. Anthropogenic sources of isoprene in Harris County, TX, USA

Point Source Emissions	**Tons/Day**	**Percent (%)**
Chemical Manufacturing	0.21714	54.46
Secondary Metal Production	0.00002	0.005
Mineral Products	0.00048	0.12
Petroleum Industry	0.02594	6.51
Fabricated Metal Products	0.00283	0.71
Printing and Publishing	0.00056	0.14
Surface Coating Operations	0.00053	0.13
Petroleum Product Storage at Refineries	0.08927	22.39
Petroleum Liquids Storage(non-Refinery)	0.05041	12.64
Organic Chemical Storage	0.00163	0.41
Organic Chemical Transportation	0.00771	1.93
Organic Solvent Evaporation	0.00218	0.55
Emissions	**Tons/Day**	**Percent (%)**
Point sources	0.6772	70.26
Mobile sources	0.2303	23.89
Area sources	0.0013	0.13
Off-road sources	0.0551	5.72
Total	**0.9639 (= 874 kg/day)**	**100**

2.2.2 Photodegradation

In air, a chemical can react with photosensitized oxygen in the form of $^{\bullet}$OH. This reaction is characterized as indirect photodegradation, and can result in a parent chemical's complete degradation. Isoprene rapidly reacts with $^{\bullet}$OH in air.

Potential $^{\bullet}$OH reaction rates with isoprene and the atmospheric chemical half-life can be calculated based on an average $^{\bullet}$OH radical concentrations. The atmospheric oxidation potential model (Meylan and Howard, 1993) calculates a rate constant of 105.14 x 10^{-12} $cm^3mol^{-1}s^{-1}$ for isoprene and an average isoprene atmospheric half-life ($t_{½}$) of 1.2 hours or 0.1 days based on a 12-hour light period in a day (the 12-hour day half-life value normalizes degradation to a standard day light period during which hydroxyl radicals needed for degradation are generated). This value is calculated using an average global $^{\bullet}$OH concentration of $1.5x10^6$ $^{\bullet}OH/cm^3$ (EPIWIN, 1999). These data indicate that indirect photodegradation can significantly contribute to the degradation of isoprene in the environment.

In comparison, direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may undergo a transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break

chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer.

An approach to assessing the potential for a substance to undergo photochemical degradation is to assume that degradation will occur in proportion to the amount of light absorbed by the chemical at wavelengths >290 nm (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, photolysis does not contribute to the degradation of isoprene in the aquatic environment.

2.2.3 Stability in Water

Results from equilibrium distribution modeling (Mackay Level I and Level III; see next Section 2.2.4) show that isoprene has the potential to partition to water at significant concentrations only when emitted to this compartment. However, the levels of isoprene that may occur in aquatic environments are unlikely to degrade by hydrolysis because it lacks a functional group that is hydrolytically reactive (Gould, 1959; Harris, 1982). Therefore, this degradative process will not contribute to the removal of isoprene from the environment.

2.2.4 Transport between Environmental Compartments

Fugacity-based multimedia modelling provides basic information on the relative distribution of a chemical between selected environmental compartments (i.e., air, soil, water, sediment, suspended sediment, and biota). Widely used fugacity models are the EQC (Equilibrium Criterion) Level I and Level III models (Mackay, 1996). These models require the input of basic physicochemical parameters (i.e., molecular weight, melting point, vapor pressure, water solubility, log K_{ow}). The Level III model also requires the input of emission rate and half-life data.

Results of the Mackay Level I environmental distribution model (Table 2) show that isoprene has the potential to partition primarily to air, with a negligible amount partitioning to water and soil. These results can be explained by isoprene's high vapour pressure, 733.3 hPa at 25°C (EPIWIN, 1999). Whereas, Level III modeling (Table 3) indicates that isoprene partitions mostly to the water compartment rather than air compartment when an equal emission rate (1000 kg/hr) to each compartment is assumed. The shorter half-life in the air compartment relative to other compartments contributes to this result. When releases occur only to each of the air and water compartments, independent of one another, isoprene is indicated in the modeling to partition primarily to those compartments, respectively. However, Level III modeling is unlikely to be representative of the ultimate disposition of isoprene because a default emission rate (1000 kg/hr) was used in the model and is not representative of actual chemical discharge.

Table 3. Environmental distribution as calculated by the Mackay (1998) Level I fugacity model

Environmental Compartment	Percent Distribution*
Air	99.92
Water	0.06
Soil	0.02
Sediment	0.00
Suspended Sediment	0.00
Biota	0.00

*Distribution is based on the following model input parameters (from Table 1):

Molecular Weight	68.12
Temperature	25° C
Log P_{ow}	2.42 (measured value)
Water Solubility	642 g/m^3 (measured value)
Vapor Pressure	733.3 hPa (measured value)
Melting Point	-145.9° C (measured value)

Table 4. Environmental distribution as calculated by the Mackay (1998) Level III fugacity model

Environmental Compartment	Percent Distribution* (equal emission rate to each compartment, 1000 kg/hr**)	Percent Distribution* (releases only to the air compartment, 1000 kg/hr)	Percent Distribution* (releases only to the water compartment, 1000 kg/hr)
Air	3.11	99.96	0.42
Water	87.72	0.02	99.34
Soil	8.96	0.02	0.00
Sediment	0.21	0.00	0.24

*Distribution is based on the following model input parameters (from Table 1):

Molecular Weight	68.12
Temperature	25° C
Log P_{ow}	2.42 (measured value)
Water Solubility	642 g/m^3 (measured value)
Vapor Pressure	733.3 hPa (measured value)
Melting Point	-145.9° C (measured value)
Degradation Half-lives (hr):	
Air	1.2
Water	120
Soil	420
Sediment	420

**Emission rate is 1000 kg/hr into each of air, water, and soil compartments.

2.2.5 Biodegradation

Isoprene was shown to biodegrade to an extent of 60.9% after 28 days based on a study that used the OECD 301F, manometric respirometry, test guideline (ExxonMobil Biomedical Sciences, Inc., 2004), but was not readily biodegradable because the replicate data exceeded the allowable range

(53 to 75%). In an OECD 301D study, isoprene biodegraded to an extent of 2 and 58% in duplicate samples after 28 days, and showed no inhibitory effect in a supplementary study. The supplementary study resulted in 64% biodegradation on day 7, using the acclimated inoculum from the initial study.

Based on scientific judgement that considered estimated aerobic biodegradation half-life values, the biodegradation half-life of isoprene in soil is estimated to range from 7 to 28 days (Howard *et al.*, 1991).

2.2.6 Bioaccumulation

Measured log bioconcentration factor (BCF) values are reported as 0.7 to 1.1 (BCF = 5.0 to 14) and 0.7 to 1.3 (BCF = 5.6 to 20) at isoprene exposure concentrations of 50 and 5 mg/L, respectively (CITI, 1992). A log BCF of 1.16 (BCF = 14.6) is calculated (EPIWIN, 1999) with respect to the log P_{ow} = 2.42. Isoprene in the aquatic environment is expected to demonstrate a low potential for bioaccumulation.

2.2.7 Other Information on Environmental Fate

Isoprene has the potential to rapidly volatilize from surface waters, based on a Henry's Law constant (HLC) representing volatility of 7,781 Pa-m^3/mole. The HLC was calculated using a water solubility of 642 mg/L, a vapor pressure of 733.3 hPa, and a molecular weight of 68.12. The volatilisation half-life of isoprene from a model river and lake is estimated to be approximately 0.8 hours and 3.3 days, respectively (EPIWIN, 1999). Isoprene is not expected to sorb significantly to organic matter in soil, sediment, and wastewater solids based on a log K_{oc} of 1.83 (EPIWIN, 1999).

The photochemical ozone creation potential (POCP) index for a chemical provides a relative measure of its reactivity or ozone forming potential. The POCP index can also provide a means of ranking volatile organic compounds (VOCs) by their ability to form ozone in the troposphere. Reported POCP indices for isoprene in northwestern Europe range from 109.2 to 117.8 (Derwent *et al.*, 1996; Derwent *et al.*, 1998), in comparison with a POCP index of 100 for ethylene, the reference substance.

Isoprene can react easily with hydroxyl radicals. The atmospheric life-time is less than 1 day. Isoprene does not have Cl- or Br-atoms. Therefore, reactive Cl- or Br-substances, which can have an adverse impact on stratospheric ozone concentration, are not formed following photochemical degradation and the ozone depletion potential of this substance is considered negligible. When considered with isoprene's relatively short atmospheric half-life, its contribution to global warming can be considered minor.

2.3 Human Exposure

2.3.1 Occupational Exposure

Isoprene is a petrochemical that is used as a chemical intermediate in contained systems. Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used. The National Institute for Occupational Safety and Health (NIOSH) estimated that 3,654 workers (578 of these are female) were potentially

exposed to isoprene in the US (from a National Occupational Exposure Survey 1981 to 1983) (NIOSH, 1989).

The following are reported occupational exposure limits:

- MAC (time weighted average) = 100 mg/m^3, Poland (RTECS)
- MAC (short-term exposure limit) = 300 mg/m^3, Poland (RTECS)
- Short term exposure limit = 40 mg/m^3, Russia (International Labour Office, 1983)
- Workplace Environmental Exposure Level (WEEL): 8-hr Time-weighted Average (TWA) = 2 ppm, United States (AIHA, 2005)

Catarrhal inflammation, subtrophic, and atrophic processes in the upper respiratory tract, as well as deterioration of olfaction were noted in isoprene rubber production workers; prevalence and degree were correlated with increasing length of service (IARC, 1994).

2.3.2 Consumer Exposure

There are no direct sales to consumers. Consequently, potential for consumer exposure will be negligible. Isoprene is used as a chemical intermediate primarily in the manufacture of polymers. Isoprene is also used for production of polymers used as food contact materials and was evaluated in this framework (Scientific Committee on Foods, 2000). Therefore exposure of consumers to this substance from the food contact materials need not be taken into account in the OECD framework. Isoprene is used in production of polymers used in paint resins, tires, footwear, moduled goods, adhesives, and motor oil viscosity improvers. An unknown percentage of unreacted monomers is present in the end-products. Possible migration rate is also unknown. The permission for use of isoprene rubber for food contact materials does not cover other consumer uses. The consumer exposure from other sources is unknown.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

The metabolism and toxicokinetics of isoprene have been thoroughly reviewed by Watson *et al.* (2001) and Csanady and Filser (2001). Species differences in the endogenous production of isoprene have been reported. Isoprene metabolism as described by Michaelis-Menten kinetics has been shown to be saturable (Bond *et al*. 1991; Dahl *et al*., 1987; and Peter *et al*., 1987). The relevant studies are summarized below.

Studies in Animals

In vivo Studies

Isoprene is produced endogenously in both rats and mice. However, there are species differences in the rate of production. In studies by Peter *et al.* (1987, 1990) endogenous production of isoprene was calculated to be 1.9 and 0.4 µmol/hr/kg in rats and mice, respectively. When untreated rats or mice were kept in a closed exposure system the concentration of exhaled isoprene was measured to be 0.8 and 0.2 ppm, respectively. Under these particular conditions the metabolic rate for endogenously produced isoprene was estimated to be about 1.6 µmol/hr/kg in rats and 0.3 µmole/hr/kg in mice. However, Filser *et al.* (1996) reinvestigated the method of Peter *et al.* (1987)

and were unable to discriminate between the endogenous compounds acetone and isoprene. By using a different technique, Filser *et al.* (1996) detected smaller (several ppb) concentrations of endogenous isoprene production in rats compared to 0.6 ppm measured in humans.

In a pharmacokinetic study conducted by Peter *et al.* (1987), both rats and mice were exposed to 5 to 1000 ppm isoprene. The rate of metabolism was directly proportional to the exposure concentration at concentrations up to 300 ppm. However, both species exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm. The maximal metabolic elimination rate in mice was determined to be 400 μmol/hr/kg or more. This shows the rate of metabolism in mice is about 2 or 3 times that found in rats (130 μmol/hr/kg).

Mice and hamsters showed more rapid and extensive metabolism to epoxide metabolites compared to rats and rabbits (Longo *et al.*, 1985). These epoxide metabolites are likely responsible for the toxic effects of isoprene and may explain why the mouse is more susceptible to isoprene toxicity.

In a study by Dahl *et al.* (1987), male F344 rats were exposed by nose-only inhalation for 6 hours to 8, 260, 1480, and 8200 ppm ^{14}C-labeled isoprene. Increasing isoprene concentrations resulted in an increased amount of retained ^{14}C metabolites; however, this was not a linear increase. The percentage of the inhaled isoprene that was metabolized decreased with increasing exposure concentration. The concentration of isoprene in the blood was below detection 1-2 minutes after termination of exposure to 8 or 260 ppm isoprene. About 75% of the total metabolites were excreted in urine, independent of inhaled isoprene concentration. After exposure to 8200 ppm, a larger percentage of the metabolites was excreted in feces than after exposure to lower concentrations. At one exposure concentration, 1480 ppm, metabolites were measured in the nose, lungs, liver, kidney, and fat, as well as in blood. A mutagenic metabolite, isoprene diepoxide, was tentatively identified in all tissues examined. Between 0.0018 and 0.031% of the inhaled ^{14}C label was tentatively identified as isoprene diepoxide in blood. The relative amount of the metabolites present in blood was highest for low concentrations of inhaled isoprene and for shorter exposure durations. The appearance of metabolites in the respiratory tract after short exposure durations together with low blood concentrations of isoprene indicated that substantial metabolism of inhaled isoprene in the respiratory tract may occur. After an approximately 6-hr exposure to 1480 ppm isoprene, the concentration of reactive metabolites in blood reached a plateau. This demonstrates that the metabolism of isoprene is saturable.

In a study by Bond *et al.* (1991), male B6C3F1 mice were exposed to 20, 200 and 2000 ppm isoprene or ^{14}C-isoprene for up to 6 hours. For all exposures, steady-state levels of isoprene were reached rapidly (i.e., within 15 to 30 minutes) after the onset of exposure. Hemoglobin adduct formation reached near-maximum between 200 and 2000 ppm which is consistent with the conclusion that pathways for metabolism of isoprene were saturated. The metabolism of isoprene was linearly related to exposure concentrations up to 200 ppm but decreased at 2000 ppm. Isoprene metabolites were present in blood after inhalation of isoprene at all concentrations studied.

In vitro Studies

Isoprene is metabolized by microsomal cytochrome P-450 dependent mono-oxygenases (Gervasi and Longo, 1990). Isoprene is metabolized by oxidation of either double bond resulting in the formation of two monoepoxides (i.e., 1,2-epoxy-2-methyl-3-butene and 3,4-epoxy-2-methyl-1-butene) as observed in liver microsomal preparations from rats, mice, rabbits, and hamsters. These monoepoxide metabolites may be hydrolyzed or conjugated with glutathione. The 3,4-epoxy-2-methyl-1-butene (minor pathway) may be further oxidized to the diepoxide, 2-methyl-1,2:3,4-diepoxide . The maximum metabolic velocity (V_{max}) of this oxidation reaction has been shown to be 6-fold higher in liver microsomes from mice and Syrian hamsters than from microsomes in rats

and rabbits (Bogaards *et al.*, 1996; DelMonte *et al.*, 1985; Gervasi and Longo, 1990; Longo et al., 1985; Small *et al.*, 1997; and Wistuba *et al.*, 1994).

Comparative studies with mammalian *in vitro* systems indicate that there are significant stereochemical and mechanistic differences among species (Small *et al.*, 1997). Enantiomers of the monoepoxide 2-(1-methylethenyl)oxirane were identified in liver microsome preparations from rats, mice, rabbits, dogs, monkeys, and humans. Rats preferentially formed (S)-2-(1-methylethenyl)oxirane compared with the (R)-enantiomer, whereas microsomes from dog, monkey, or male human preferentially formed (R)-2-(1-methylethenyl)oxirane. Metabolites from isoprene incubated with human female microsomes and with mouse and rabbit microsomes did not show enantioselectivity.

Studies in Humans

In vivo Studies

Isoprene is produced endogenously in humans, probably from mevalonic acid (Deneris *et al.*, 1984), a precursor of cholesterol biosynthesis. The endogenous production rate of isoprene was calculated to be 0.15 μmol/kg/hour (Hartmann and Kessler, 1990). Concentrations in human blood range between 15-70 nmol/L (mean = 37 nmol/L), but were <1 nmol/L in other animal species including rats, rabbits, and dogs (Cailleux *et al.*, 1992). Isoprene is also found in human breath at concentrations in the range of 10-30 nmol/L (Cailleux and Allain, 1989). The quantity of isoprene exhaled daily per individual was estimated to be 2-4 mg (Gelmont *et al.*, 1981).

In a preliminary report based on a study in 5 male and 1 female human volunteer exposed to isoprene via a closed spirometer system, the pharmacokinetics of isoprene in humans was reported to more closely resemble rats than mice (Hartmann and Kessler, 1990). These data were reported in more detail by Filser *et al.* (1996).

By generating data from human exposures at 0, 8, or 40 ppm isoprene, Filser et al. (1996) determined endogenous isoprene production for a 70 kg man to be 23.8 μmol/hr and the rate of metabolism to be 0.34 μmol/hr/kg. A toxicokinetic model predicted 3.4 mg of isoprene would be exhaled in a 24-hr period, which is in direct agreement with determinations made by other authors (Conkle *et al.*, 1975: 0.36 to 9.36 mg/24-hr; Gelmont *et al.*, 1981: 2 to 4 mg/24-hr). By using his toxicokinetic model to compare the data in mice (Bond *et al.*, 1991) and rats (Dahl *et al.,* 1987) with that obtained from humans (Filser *et al.*, 1996; Hartmann and Kessler, 1990), Filser *et al.* were able to show isoprene metabolism leading to production of both monoepoxides is up to 3.8 times faster in mice than in rats. At exposure concentrations up to 50 ppm, the rate of metabolism at steady state is 14 times faster in mice and approximately 8 times faster in rats than in humans.

In vitro Studies

The metabolism of isoprene and two isoprene monoepoxides was investigated with microsomes from cell lines expressing eight different human cytochrome P450 enzymes and with liver microsomes from humans, rats, and mice (Bogaards *et al.*, 1996). The single human enzymes were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, CYP3A4. CYP2E1 showed the highest rates of formation for the two isoprene monoepoxides and was the only enzyme showing detectable formation of the isoprene diepoxide. Both isoprene monoepoxides were oxidized by CYP2E1 to the diepoxide at similar enzymatic rates. In order to determine the relative role of CYP2E1 in hepatic metabolism, isoprene as well as the two monoepoxides were incubated with a series of ten human liver microsomal preparations in the presence of the epoxide hydrolase inhibitor, cyclohexene oxide. Isoprene monoepoxide metabolism showed a significant correlation with CYP2E1 activity, determined as chlorzoxazone 6-hydroxylation. It was concluded that CYP2E1 is the major enzyme involved in hepatic metabolism of isoprene and the isoprene monoepoxides *in vitro*.

In order to investigate species differences with regard to the role of epoxide hydrolase in the metabolism of isoprene monoepoxides, the epoxidation of isoprene by human liver microsomes was compared to that of mouse and rat liver microsomes. The amounts of monoepoxides formed as a balance between epoxidation and hydrolysis, was measured in incubations with and without the epoxide hydrolase inhibitor cyclohexene oxide. Inhibition of epoxide hydrolase resulted in similar rates of monoepoxide formation in mouse, rat and man. However, without inhibitor the total amount of monoepoxides present at the end of the incubation period for mouse liver microsomes was twice as high as for rat and 15 times as high as for human liver microsomes (Bogaards *et al.*, 1996).

Conclusion

Clear differences exist in the toxicokinetics, metabolism and distribution of isoprene among species. Differences in metabolism and reactivity of the metabolites may contribute to the significant differences in toxicological response to isoprene observed between species.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

An acute inhalation exposure study was conducted by Shugaev (1969) to determine the concentrations of hydrocarbons such as isoprene in various tissues at lethal exposure concentrations. In this pre-GLP study minimal detail is provided. The LC_{50} values reported in this study are as follows:

Rat LC_{50} (4 hour) = 180 mg/L or 64,620 ppm (confidence limits 130 to 181 mg/L)

Mouse LC_{50} (2 hour) = 157 mg/L or 56,363 ppm (confidence limits 129 to 252 mg/L)

An acute isoprene inhalation exposure study was also conducted by Gostinskii (1965). In this pre-GLP study conducted in mice, few study details are provided. However, the following LC_{50} values are reported:

Female Mouse LC_{50} (2 hour) = 148 mg/L or 53,121 ppm (confidence limits 144-153 mg/L)

Male Mouse LC_{50} (2 hour) = 139 mg/L or 49,891 ppm (confidence limits 135-143 mg/L)

Oral

An acute oral toxicity study was conducted in Wistar rats. In this study, 15 animals per sex were administered isoprene in oil. The oral LD50 was determined to be 2043 to 2210 mg/kg. No other details were provided (Kimmerle and Solmecke, 1972).

Conclusion

The acute toxicity data on isoprene are limited. However, using a weight-of-evidence approach, the available data suggest that isoprene has a low order of acute toxicity in animals by the inhalation and oral routes of exposure.

3.1.3 Irritation

Studies in Animals

Skin Irritation

Although three studies are cited in the dossier, details are only available for the study conducted by Kimmerle and Solmecke (1972). In this skin irritation study, the skin of two New Zealand White rabbits was painted twice per day for 5 consecutive days with 100% isoprene. Reversible erythema was observed. Using a weight-of-evidence approach, the data suggest that isoprene has a low potential for skin irritation.

Eye Irritation

In a non-GLP study conducted by Mamedov (1979), isoprene was reported to cause eye irritation in rats. However, no study details are provided.

Respiratory Tract Irritation

A repeated dose inhalation study conducted in mice and rats exposed up to 7000 ppm (19,503 mg/m^3) isoprene for 6 hours/day, 5 days/week for 13 weeks showed no gross microscopic lesions in the respiratory tract of rats and female mice (Melnick *et al.*, 1994). In male mice degeneration of the olfactory epithelium was observed at 7000 ppm, but not at lower concentrations. The NOAEL in this study was 2200 ppm (6129 mg/m^3).

Studies in Humans

Skin Irritation

It has been reported that isoprene is irritating to the skin, eyes and mucous membranes (Lewis, 1996).

Eye Irritation

Vapors of isoprene have been reported to produce slight irritation of the eyes and upper respiratory tract. Liquid isoprene may irritate the eyes (Chemical Hazard Response Information System, 2001)

Respiratory Tract Irritation

Gostinskii (1965) reported that human volunteers experienced slight irritation of the upper respiratory tract at an isoprene concentration of 57 ppm (160 mg/m^3). However, the details in this pre GLP study are limited therefore, the reliability of the study cannot be assessed.

IARC reports that in isoprene rubber production workers, subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract were observed. Prevalence and degree were correlated with increasing length of service (IARC, 1994).

Conclusion

Using a weight-of-evidence approach, isoprene appears to have a low potential for skin and respiratory tract irritation. However, the eye irritation potential of isoprene in animals or humans cannot be determined as no data are available for this endpoint.

3.1.4 Sensitization

No animal or human data are available for this endpoint.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

A 2-week repeated dose inhalation study was conducted in both mice and rats (Melnick *et al.*, 1990). In this study, F344 rats and B6C3F1 mice (20 animals/sex/group/species) were exposed by whole-body inhalation to isoprene at concentrations of 0, 438, 875, 1750, 3500, or 7000 ppm (i.e., 0; 1220; 2438; 4876; 9751; or 19,503 mg/m^3) for 6 hours/day, 5 days/week for two weeks. Ten animals/sex/group/species were used for clinical pathology evaluations after 4 exposures in rats or 5 exposures in mice. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed-effect level was found. The results for each species are described separately below.

In rats, no treatment-related changes in survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or incidences of gross or microscopic lesions were observed at any dose following the 2-week exposure. Thus, in this study, the NOAEL for rats was 7000 ppm (19,503 mg/m^3).

Exposure of the B6C3F1 mice to isoprene for 2-weeks did not produce mortality at any of the doses tested. A 15% reduction in body weight was observed in the 7000 ppm group of male mice. Exposure to isoprene did not cause reduction in mean body weight gain of female mice. Dose-related increases in mean liver weight/body weight ratios and decreases in relative thymus, spleen, and testis weights were observed in mice exposed to isoprene compared to controls. Organ weight changes were observed in both male and female mice.

In blood samples of mice exposed for 5 consecutive days to isoprene, mean red blood cell counts, hemoglobin concentrations, and volume of packed red cells were reduced in all exposure groups when compared to controls. These changes were not dose related nor accompanied by increases in reticulocyte counts or polychromatic erythrocytes. Similar hematologic changes were observed in male and female mice. The lack of exposure-related changes in serum chemistry parameters in mice indicates that the hepatic and renal systems were not adversely affected in this species after 5 days of exposure to isoprene.

Microscopic changes in the thymus, testes, nasal cavity and forestomach were observed in male mice following exposure to isoprene for 2 weeks. Microscopic forestomach lesions were also observed in exposed female mice. Thymic atrophy in male mice exposed to 7000 ppm was characterized by a decrease in cellularity of the cortex. Minimal testicular atrophy was observed in mice exposed to 7000 ppm isoprene. Diffuse liver changes consistent with highly glycogenated hepatocytes were observed to similar degrees in all dose groups of exposed male mice. Olfactory epithelial degeneration was observed at 1750, 3500, and 7000 ppm isoprene; the severity of the nasal lesions increased with increasing concentrations of isoprene.

Epithelial hyperplasia of the forestomach was seen in all groups of male and female mice exposed to isoprene. The severity of the lesion was relatively consistent throughout the exposure groups; a no observable-effect level for this lesion was not achieved at the exposure concentrations used in this study. The changes in spleen weights in mice exposed to isoprene were not associated with histopathological alterations in this organ. The significance of the observed changes in the liver of male mice but not female mice, is unclear.

In conclusion, this study demonstrated that there is a clear species difference in the susceptibility of rats and mice to isoprene exposure. In rats, there were no observable toxicological effects at any

dose following the 2-week exposure. The NOAEL for rats was 7000 ppm (19,503 mg/m^3). However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest concentration tested, i.e., 438 ppm (1220 mg/m^3). Thus, in this study, the LOAEL for mice was 438 ppm (1220 mg/m^3).

A 13-week repeated dose whole-body inhalation study was conducted in mice and rats (Melnick *et al.*, 1994). In this study, F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) were exposed to 0, 70, 220, 700, 2200, and 7000 ppm (i.e., 0; 195; 613; 1950; 6129; 19,503 mg/m^3) isoprene, 6 hours/day, 5 days/week for 13 weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats and mice were sacrificed and evaluated histopathologically. Organ weights were also recorded.

In rats, no exposure-related effects were observed at any dose for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions.

In mice, there were no effects on survival, body weight gain, or clinical signs of toxicity. However, the male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. Focal epithelial hyperplasia of the forestomach was also observed in both males and females exposed to 700 ppm or higher. Degeneration of the olfactory epithelium was observed only in male mice at the highest concentration (i.e., 7000 ppm). Cytoplasmic vacuolization of hepatocytes due to glycogen accumulation was also observed in male mice at the two highest dose levels (i.e., 2200 and 7000 ppm). However, this effect was only statistically significant at the 7000 ppm dose level.

In conclusion, in this study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/m^3) for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/m^3) and higher. This 13-week repeated dose inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed that mice are more susceptible to the effects of isoprene than rats.

Non-neoplastic effects were also observed following isoprene exposure in both the 26-week and the 104-week lifetime study conducted in mice and rats. These non-neoplastic effects are described below.

In the 26-week whole-body inhalation exposure study (Melnick et al., 1994), groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0, 70, 220, 700, 2200, or 7000 ppm (0; 195; 613; 1950; 6129; 19,503 mg/m^3) isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically.

In rats, the only non-neoplastic effect observed following 26 weeks of exposure, was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm (19,503 mg/m^3)isoprene group. No other gross or histopathological lesions in rats were attributed to isoprene exposures.

In mice, survival was reduced in the 7000 ppm group following the 26-week exposure period. In addition, near the end of the exposure period, abnormal posture and impaired hindlimb function were observed primarily in the 7000 ppm group; however, during the recovery period these clinical

signs subsided and affected animals gradually returned to a clinically normal state. Hindlimb grip strengths were significantly less in mice in the 220 ppm and higher exposure groups compared to controls. Hindlimb grip strengths remained lower than controls at day 2 of the recovery period. By 4 weeks postexposure, hindlimb grip strengths of exposed mice were generally similar to those of controls.

Although no treatment-related histopathological changes were detected in the lungs of isoprene-exposed mice at the end of the 26-week exposure period, an increased incidence of alveolar epithelial hyperplasia was observed in the 700 ppm and higher exposure groups following the 26-week recovery period. In addition, at the end of the 26-week exposure, focal hyperplasia of the forestomach epithelium, was observed in most mice in the 700, 2200 and 7000 ppm exposure groups. Following the 26-week recovery period, the incidence of forestomach hyperplasia was greater in the 700 ppm and higher exposure groups than in the controls.

Mild to minimal olfactory epithelial degeneration in the nasal cavity was also observed in all mice in the 7000 ppm exposure group after 26 weeks of exposure to isoprene. At the end of the 26-week recovery period, the incidence of mild to moderate olfactory epithelial degeneration was significantly elevated in the 220 ppm and higher exposure groups.

Exposure-related decreases in testis weight were observed in mice following 26 weeks of exposure to isoprene but not after the recovery period. Testicular atrophy was also observed in male mice exposed to 7000 ppm isoprene for 26 weeks but this effect was not observed after the recovery period.

Lastly, minimal degeneration of the spinal cord white matter was evident in mice exposed to 7000 ppm isoprene for 26 weeks; however, after the 26-week recovery period, the incidence of spinal cord degeneration was significantly increased in all exposure groups. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above. In a chronic oncogenicity study, $B6C3F_1$ mice were exposed to isoprene by inhalation for either 4 or 8 hours/day, 5 days/week for 20, 40 or 80 weeks (Placke *et al.*, 1996). Twelve groups of 50 male $B6C3F_1$ mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm (i.e., 0, 28, 195, 390, 780, 1950, 6129 mg/m^3) of isoprene vapor for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period, leading to a total planned study length of 104 weeks. Female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80 week exposure period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105.

With respect to nonneoplastic lesions, there were no apparent effects on motor function and no exposure-related lesions in the spinal cord at any concentration. This is in sharp contrast to what was observed in the NTP subchronic study where partial hindlimb paralysis and spinal cord degeneration was observed in mice exposed to 70 ppm (195 mg/m^3) for 6 months.

A chronic inhalation oncogenicity study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220, 700, or 7000 ppm (613; 1950; 19,503 mg/m^3) isoprene by inhalation, 6 hours per day, 5 days per week, for 104 weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Non-neoplastic findings in this study included renal tubule hyperplasia and splenic fibrosis. The incidence of renal tubule hyperplasia was significantly greater in males exposed to 7000 ppm (19,503 mg/m^3) isoprene than in the chamber controls. In addition, the severity of kidney nephropathy was slightly increased in 7000 ppm (19,503 mg/m^3) males when compared to chamber

controls. The incidences of splenic fibrosis in 700 ppm (1950 mg/m^3) and 7000 ppm (19,503 mg/m^3) males were significantly greater than that in the chamber control group.

Studies in Humans

Catarrhal inflammation, subtrophic and atrophic processes in the upper respiratory tract and deterioration of olfaction were noted in isoprene rubber production workers. Prevalence and degree were correlated with increasing length of service (IARC, 1994).Conclusion

High quality repeated dose studies demonstrate clear species differences between rats and mice in susceptibility to isoprene. For example, in rats, there were no observable toxicological effects at any dose following the 2-week repeated dose exposure. However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest level tested, i.e., 438 ppm (1220 mg/m^3). Similarly, in the 13 week study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/m^3) isoprene for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/m^3) and higher. This 13-week repeated dose inhalation study confirmed the species difference between rats and mice in susceptibility to isoprene. The fact that mice are more susceptible to isoprene than rats was further demonstrated in both the 26-week and 104-week isoprene inhalation studies.

3.1.6 Mutagenicity

Isoprene has been tested for mutagenic activity in both *in vivo* and *in vitro* test systems. Anderson (2001) reviewed the genetic toxicity of isoprene. The critical studies are discussed below.

In vivo Studies

An in vivo Sister Chromatid Exchange (SCE) study and a Mammalian Bone Marrow Chromosomal Aberration study were conducted in B6C3F1 mice by Tice et al. (1988). In the SCE study, fifteen male B6C3F1 mice per group were exposed for 12 days, 6 hours/day to 0, 438, 1750, or 7000 ppm (0; 1220; 4876; 19,503 mg/m^3) of isoprene by inhalation. Positive control substances were not included in the study. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours prior to sacrifice on the following day, the animals received an intraperitoneal injection of colchicine.

For analysis of SCE, 5 mice per exposure group were euthanized 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group.

For analysis of chromosomal aberrations, 10 mice per exposure group were killed 17-20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for aberrations from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1000 cells/sample was used to calculate the mitotic index.

Exposure to isoprene for 6 hours/day at 0, 438, 1750, or 7000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells at all three dose levels (4.40 at 0 ppm, 14.84 at 438 ppm, 11.61 at 1750 ppm, and 13.98 at 7000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other. The lack of significant difference in

SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species. There were no significant clinical signs or mortality throughout the study.

In conclusion, in this study, isoprene was found to be genotoxic to bone marrow cells *in vivo* as indicated by the significant increase in the frequency of SCEs in bone marrow cells at all three dose levels. It was also found to be cytotoxic to mouse bone marrow as indicated by a significant lengthening of the bone marrow average generation time (AGTh) and the dose-dependent decline in the percentage of peripheral blood polychromatic erythrocytes (i.e., %PCE), a measure of the overall rate of erythropoiesis. In this study exposure to 0, 438, 1750 and 7000 ppm (0; 1220; 4876; 19,503 mg/m^3) isoprene resulted in an AGT (h) of 11.68, 12.98, 12.73 and 13.72, respectively. The %PCE following exposure to 0, 438, 1750 and 7000 ppm (0; 1220; 4876; 19,503 mg/m^3) isoprene was 3.91, 3.00, 2.87 and 1.64, respectively.

Exposure of mice to isoprene for 6 hours/day at concentrations of 0, 438, 1750, or 7000 ppm (0; 1220; 4876; 19,503 mg/m^3) for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations in bone marrow cells (Tice et al.,1988). The incidence of bone marrow cells with chromosomal aberrations was slightly elevated in the exposed groups compared to the control but these increases were not statistically significant. Mitotic index data indicated no significant change in the percentage of bone marrow cells engaged in division, although the 7000 ppm group was slightly increased compared to the controls. Analysis of the average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group.

In conclusion, although the incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days were slightly elevated at all dose groups compared to controls, none of the increases were statistically significant.

A Mammalian Erythrocyte Micronucleus Test was conducted in B6C3F1 mice by Tice et al. (1988). In this study, 15 male B6C3F1 mice per group were exposed by inhalation to isoprene at concentrations of 0, 438, 1750, and 7000 ppm (0; 1220; 4876; 19,503 mg/m^3), 6 hours/day for 12 days. Approximately 24 hours following the last exposure, peripheral blood samples were obtained from each animal by tail snip, air-dried immediately and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity.

Exposure to isoprene induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure concentrations tested. There was also a dose-related decrease in the percentage of PCEs, a measure of the rate of erythropoiesis. There were no significant clinical signs or mortality throughout the study.

In conclusion, isoprene was genotoxic to mouse bone marrow *in vivo*. A decrease in the percentage of PCEs was also observed which suggests that erythropoiesis was also being suppressed.

A rat lung fibroblast Micronucleus Test was conducted in Fischer 344 rats by the National Toxicology Program (1997). Groups of 10 male and 10 female rats per group were exposed for 4 weeks to 0, 220, 700, or 7000 ppm (0; 613; 1950; 19,503 mg/m^3) isoprene by inhalation for a total of 17 to 19 exposures. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained with acridine orange and 1000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.

There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.

In conclusion, isoprene was not genotoxic in this study. No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.

In vitro Studies

Isoprene was tested in an Ames assay in four strains of *Salmonella typhimurium* (i.e., TA98, TA100, TA1535, TA1537) with and without metabolic activation (Mortelmans et al., 1986). The preincubation modification of the Salmonella assay was used to test isoprene in these four different strains of Salmonella in the presence and absence of Aroclor 1254-induced rat and hamster liver S-9. Five dose levels plus control were tested (i.e., 0, 100, 333, 1000, 3333, and 10,000 μg/plate) with three plates per dose level. The high dose (10,000 μg/plate) was limited by toxicity. Concurrent positive controls were also tested with and without metabolic activation. The assay was repeated less than one week after completion of the initial test. All positive control substances produced at least a two-fold or three-fold increase in revertant colonies in their respective strains when compared with the vehicle controls. In this study, isoprene was not mutagenic in any of the four strains of Salmonella tested either in the presence or absence of Aroclor-induced rat or hamster liver S9. Concentrations of 0.25 to 25% isoprene monomer was subjected to Ames mutagenicity testing using vapor phase exposures (Huntington Life Sciences, Ltd., 2003a). To investigate possible species differences in metabolism, liver enzymatic preparations (S-9 and microsomes) were employed from uninduced B6C3F1 male mice. A positive control, vinyl chloride, provided evidence of both the mutagenic capabilities of the bacteria as well as activity of the liver enzymes. Salmonella strains TA1535, TA1537, TA98, and TA100 were employed as were E. coli WP2 uvrA (pKM101) bacteria. Criteria for a positive response included (a) evidence of dose-responsiveness, and (b) an increase in revertants of treated plates versus controls of 2 times for all strains except TA1535 and TA1537, which required a 3-fold increase. The maximal increase of revertant rates observed in isoprene-exposed bacteria was 1.7 for the TA1535 strain, and 1.6 in E. coli, both in the presence of S-9 activation. Vinyl chloride induced increases in revertant rates in these assays of 29- and 5.7-fold relative to negative controls, respectively. These results for isoprene indicate negligible mutagenic activity towards bacteria under the stated test conditions.

Isoprene was tested in an *in vitro* Sister Chromatid Exchange (SCE) assay in mammalian cells (Galloway et al., 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of SCEs both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. The doses tested were 50, 160, 500, and 1600 μg/ml (without S9) and 160, 500, 1600, and 5000 μg/ml (with S9). A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Fifty 2nd division metaphase cells were scored for frequency of SCEs/cell from each dose level. Isoprene was not genotoxic in this study as no increases in SCEs were noted in the cultured CHO cells treated with isoprene, with or without S9.

Isoprene was also tested in an *in vitro* Mammalian Chromosomal Aberration Test (Galloway et al., 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and three doses of isoprene. The doses tested were 1600, 3000 and 5000 μg/ml. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same

person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level.

The positive control substances produced statistically significant increases in the percentages of aberrant cells when tested with or without activation when compared to the vehicle controls. Isoprene was not genotoxic in this study as no increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.

Conclusion

Isoprene was genotoxic to mouse bone marrow *in vivo*. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and micronuclei in the bone marrow . However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats. Isoprene was not genotoxic in any of the *in vitro* assays conducted, including those for bacterial mutation or for sister chromatid exchanges or chromosomal aberrations in exposed Chinese Hamster Ovary Cells.

3.1.7 Carcinogenicity

In vivo Studies - Inhalation

A. Melnick *et al.* (1994)

A 26-week inhalation exposure study was conducted with isoprene in F344 rats and B6C3F1 mice (Melnick *et al.*, 1994). In this study, groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0, 70, 220, 700, 2200, or 7000 ppm (i.e., 0; 195; 613; 1950; 6129; or 19,503 mg/m^3) isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically.

Interstitial cell hyperplasia of the testis was observed in male rats after 26 weeks of exposure to 7000 ppm isoprene; following the 26-week recovery period, the only effect in rats was a marginal increase in benign testicular interstitial cell tumors in the 7000 ppm group. The survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and Harderian gland were significantly increased following the 26-week exposure and 26-week recovery periods at 700 ppm and higher exposures. However, in most cases, the carcinogenic effects of isoprene produced at 700 ppm (1950 mg/m^3) or 2200 ppm (6129 mg/m^3) were not very different from those at 7000 ppm (19,503 mg/m^3) (i.e., there was no clear dose-response). This is probably the result of metabolic saturation. Metabolic saturation has been shown to limit the production of epoxide intermediates at isoprene expoures greater than approximately 1500 ppm (4179 mg/m^3).

Isoprene was carcinogenic to the liver, lung, forestomach and Harderian gland of male mice after 26 weeks exposure and 26 weeks recovery. In contrast, the only effect observed in male rats was a marginally increased incidence of benign testicular adenomas at the highest exposure level (7000 ppm).

B. Placke *et al.* (1996)

In a chronic oncogenicity study, twelve groups of 50 male B6C3F$_1$ mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm (i.e., 0, 28, 195, 390, 780, 1950, or 6129 mg/m^3) isoprene vapor by

inhalation for 4 or 8 hours/day, 5 days/week for 20, 40 or 80 weeks. This was followed by a holding period, leading to a total planned study length of 104 weeks (Placke *et al.*, 1996). In this same study, female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80 week exposure period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105. Complete histopathology examinations were performed on organs and tissues from all study animals. There was a concentration-related effect on survival with around 50% or fewer of those exposed to >280 ppm for 80 weeks surviving at 95 weeks.

Isoprene exposure produced an increase in histiocytic sarcomas and in neoplasms of the liver, lung, Harderian gland and forestomach of male mice. The incidence of hepatocellular adenomas and carcinomas were increased (significantly at exposure concentrations > 140 ppm), as were hemangiosarcomas and histiocytic sarcomas of the liver. Metastases of hepatocellular carcinomas to the lung were also more prominent in animals receiving higher exposures. Some of these primary and metastatic liver tumors appeared to be more anaplastic and aggressive in their growth as compared to the spontaneous liver tumors in controls.

Primary alveolar/bronchiolar adenomas and carcinomas were significantly increased in incidence in animals receiving 700 ppm for 80 weeks (i.e., 5600 ppm-weeks), 2200 ppm for 40 weeks (i.e., 88000 ppm-weeks), and 2200 ppm for 80 weeks (i.e., 176,000 ppm-weeks). Several lung carcinomas in exposed mice were locally invasive to the mediastinal and thoracic area, and in five cases metastasized to the liver. The lung was also the metastatic site for Harderian gland carcinomas, and two metastatic squamous cell carcinomas from the forestomach. Histiocytic sarcoma of the lung was also slightly more prevalent in isoprene-exposed mice than in controls.

The incidence of Harderian gland adenomas was significantly increased as the isoprene exposure concentration increased. Harderian gland carcinomas were not as numerous as adenomas but were present in the higher concentration groups. These carcinomas were diagnosed generally by the presence of foci or nodules of Harderian gland cells in the lung parenchyma or by evidence of Harderian cells in veins or lymph vessels leaving the tumor.

Squamous cells carcinomas of the stomach were present in six mice exposed to over 5600 ppm-weeks total exposure. Most were highly invasive locally and two metastasized to the lung. Squamous papillomas of the forestomach were only found in male mice in the highest exposure groups, i.e.: 56,000 ppm-weeks, 88,000 ppm-weeks, and 176,000 ppm-weeks.

Exposed mice also had a slightly increase incidence of hemangiosarcomas in the spleen and heart compared to controls. Cardiac hemangiosarcomas are very rare in B6C3F1 mice. A review of historical tumor incidence data shows no evidence of cardiac hemangisarcomas among 658 control B6C3F1 mice in recent 2-year inhalation studies.

Female mice were exposed to lower concentrations of isoprene than males. Neoplastic lesions that may have been exposure-related were hemangiosarcomas in the spleen, Harderian gland adenomas, and adenomas of the pituitary gland (pars distalis). Since the number of actual neoplasms in these organs was small in the female mice, historical incidences were considered (National Institute of Environmental Health Sciences, Research Triangle Park, NC). In 654 control mice from various inhalation studies, the number of splenic hemangiomas and/or hemangiosarcomas was four (0.61%), suggesting that the four hemangiosarcomas in the 70 ppm female (8%) may have been related to isoprene exposure. From the same data set, the historical incidence of Harderian adenomas was 22/661 (3.33%), with a range of 0 to 16% and the historical incidence of pituitary adenomas was 127/629 (20.19%), with a range of 2 to 44%. Thus, the relationship of these latter

two neoplasms to the isoprene exposure is only equivocal, considering the variability in the incidence of these lesions.

In this study, there appeared to be a well documented threshold for oncogenic effects following isoprene exposure in mice, which varied slightly by organ and tumor type. For male mice, the LOEL appeared to be 700 ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 ppm for Harderian gland tumors. For female mice, the LOEL appeared to be 70 ppm for total non-liver, non-lung adenomas and possibly for hemangiosarcomas.

In summary, these results indicate that concentration, length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. For example, it appears that exposure concentration has a greater impact on tumor rates than weeks of exposure (Cox *et al.*, 1996). In summary, the same cumulative exposure could be more or less damaging, depending upon how it was administered over time.

C. NTP (1999)

A chronic inhalation oncogenicity study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220, 700, or 7000 ppm (613; 1950;19,503 mg/m3) isoprene by inhalation, 6 hours per day, 5 days per week, for 105-() weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study. Upon study termination, histopathological evaluations were performed on all major organ tissues from all study animals.

Exposure-related increases in the incidences of mammary gland fibroadenoma, and of fibroadenoma or carcinoma (combined), occurred in male rats in all exposure groups. Mammary gland fibroadenoma is considered to be a very rare tumor in male rats. The incidences of fibroadenoma in 7000 ppm males and all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of fibroadenoma in all exposed groups of males and females and of multiple fibroadenoma in 7000 ppm males and in all groups of exposed females exceeded the historical control ranges.

The incidences of renal tubule adenoma in 700 and 7000 ppm males and the incidence of renal tubule hyperplasia in 7000 ppm males were significantly greater than those in the chamber controls. In addition, there was an exposure-related increase in the incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in males exposed at 700 and 7000 ppm, the incidences being statistically significantly greater than those in the chamber controls. The incidences of interstitial cell adenoma in 700 and 7000 ppm males exceeded the historical control range. Several rare neoplasms including benign astrocytoma, malignant glioma, and malignant medulloblastoma, granular cell tumor and meningeal sarcoma were observed in the brain of exposed female rats. The neoplasms rarely occur in historical chamber controls. However, the fact that they are of different cell types makes it difficult to determine if they are truly exposure-related.

In summary, isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the carcinogenicity of isoprene in rats is, at most, limited. In spite of this, the NTP concluded that under the conditions of this 2-year inhalation study, there was clear evidence of carcinogenic activity of isoprene in male F344/N rats based on increased incidences of mammary gland neoplasms, renal tubule adenoma,

and testicular adenoma. They also concluded that there was some evidence of carcinogenic activity of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene. In summary, based on the results of the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as "reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens.

Conclusion

There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, on the other hand, there were no significant increases in the incidence of malignant tumors, but isoprene exposures were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types, makes it difficult to determine if they are truly exposure-related.

In summary, based on the results of the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as "reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens. Based on their review, IARC (1999) has classified isoprene as a Group 2B carcinogen, i.e., possibly carcinogenic to humans.

3.1.8 Toxicity for Reproduction

The reproductive toxicity of isoprene has been reviewed by Anderson (2001). Relevant studies are discussed below.

Developmental Toxicity

In a well conducted NTP (National Toxicology Program, 1989) study, female Swiss CD-1 mice and Sprague-Dawley rats were exposed to 0, 280, 1400, or 7000 ppm (i.e., 0; 780; 3900; or 19,503 mg/m^3) isoprene for 6 hours/day, 7 days/week on gestational days 6-17 in mice or gestational days 6-19 in rats.

In rats, there was no adverse effect on the dam or offspring at any dose level and there was no increase in malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm. Thus, in rats 7000 ppm was the NOAEL for both maternal and developmental toxicity.

In mice, 7000 ppm isoprene significantly reduced maternal weight gain and uterine weight. Developmental toxicity was evident in mice as a statistically significant reduction in fetal bodyweight was observed at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. Although there was no significant increase in the incidence of malformations, two fetuses with cleft palate were found, one in each of the two highest exposure groups (i.e., 1400 ppm and 7000 ppm). Cleft palate, however, is a common spontaneous finding in mice that occurs in response to maternal stress, and is not generally regarded as a manifestation of developmental toxicity in this species. Similarly, although increased incidences of variations (i.e., supernumerary ribs) were observed in the exposed groups, this skeletal variation is also considered as a secondary non-specific consequence of maternal toxicity.

In summary, in this study, 1400 ppm (3900 mg/m^3) was the NOAEL for maternal toxicity in mice. A NOAEL developmental toxicity could not be determined in this study because effects, i.e.,

reduction in fetal bodyweight, were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/m^3).

Reproductive Toxicity

No guideline reproductive studies have been conducted with isoprene. Histopathology of the reproductive organs was evaluated in a 13-week repeated dose inhalation study conducted in F344 rats and B6C3F1 mice at target concentrations up to 7000 ppm (19,503 mg/m^3)for 6 hours/day, 5 days/week for 13 weeks (Melnick et al., 1994). No exposure-related effects were observed in rats. In mice, testicular weight was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice.

Sperm motility and vaginal cytology were performed on all rats and mice exposed to 0, 70, 700, or 7000 ppm (0; 195; 1950; 19,503 mg/m^3) of isoprene in the 13-week study. Male mice in the 700 and 7000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days). Mice exhibited significant effects at 700 ppm (1950 mg/m3) or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility.

Conclusion

Inhalation exposure of pregnant rats and mice to isoprene at up to 7000 ppm (19,503 mg/m^3) produced maternal and developmental toxicity in mice but not in rats. In mice, maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm). Significant reductions in fetal bodyweight were observed at the 280 ppm (780mg/m^3) dose level for female fetuses and at the 1400 ppm (3900 mg/m^3) level for male fetuses. Thus, in this study, 1400 ppm (3900 mg/m^3) was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/m^3). In less robust reproductive toxicity evaluations, conducted as part of an NTP subchronic inhalation toxicity study, no significant effects were observed after histopathological evaluations of reproductive organs in rats except slight changes in the testis at the highest exposure level (7000 ppm (19,503 mg/m^3)). However, significant effects were observed in mice exposed to isoprene concentrations of 700 ppm (1950 mg/m^3) and higher, including increased estrous cycle length and testicular atrophy as well as decreased epididymal weight. In addition, decreased sperm head count, sperm concentration, and sperm motility were also observed.

3.2 Initial Assessment for Human Health

The available data suggest that isoprene has a low potential for acute toxicity and for skin and respiratory tract irritation. Based on structure-activity relationships, the potential for dermal or respiratory tract sensitization is considered low. However, no data are available for this endpoint. The data for other endpoints suggest that there are marked species differences between the metabolism and toxicity of isoprene in mice versus rats. Mice metabolize isoprene more readily and are more susceptible to isoprene toxicity than rats. A physiological toxicokinetic model has been developed for inhaled isoprene in mice, rats, and humans, taking into account published or assumed kinetic parameters. On the basis of this model, at human exposure conditions (up to 50 ppm [140 mg/m^3]), rates of metabolism are about 14-times faster in mice and about 8-times faster in rats than in humans.

Four repeated-dose studies (i.e., 2-week, 13-week, 26-week, and 2-year) were conducted with isoprene. No observable toxicological effects were seen in rats following exposure to isoprene for 2

weeks at doses up to 7000 ppm. In mice, however, a 2-week exposure to isoprene induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest concentration tested, i.e. 438 ppm (1220 mg/m^3). In the 13-week study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/m^3). However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/m^3) and higher. In the 26-week study, the only treatment-related effects observed in rats were slight increases in the incidence of interstitial cell adenoma of the testis following exposure to 7000 ppm (19,503 mg/m^3). In mice, however, repeated exposure for 26 weeks to isoprene at concentrations of 700 ppm (1950 mg/m^3) and higher produced malignant neoplastic lesions in the liver, lung, forestomach and Harderian gland of male mice.

Non-neoplastic effects were also observed following isoprene exposure in both the 26-week and the 104-week lifetime study conducted in mice and rats. In rats, following 26 weeks of exposure, the only non-neoplastic effect observed was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm (19,503 mg/m^3) group. However, in mice, a plethora of non-neoplastic effects were observed. In mice, survival was reduced in the 7000 ppm (19,503 mg/m^3) group following the 26-week exposure period. In addition, near the end of the exposure period, abnormal posture and impaired hindlimb function were observed primarily in the 7000 ppm (19,503 mg/m^3) group; however, during the recovery period these clinical signs subsided and affected animals gradually returned to a clinically normal state. Hindlimb grip strengths were significantly less in mice in the 220 ppm (613 mg/m^3) and higher exposure groups compared to controls. Hindlimb grip strengths remained lower than controls at day 2 of the recovery period. By 4 weeks postexposure, hindlimb grip strengths of exposed mice were generally similar to those of controls.

Although no treatment-related histopathological changes were detected in the lungs of isoprene-exposed mice at the end of the 26-week exposure period, an increased incidence of alveolar epithelial hyperplasia was observed in the 700 ppm (1950 mg/m^3) and higher exposure groups following the 26-week recovery period. In addition, at the end of the 26-week exposure, focal hyperplasia of the forestomach epithelium, was observed in most mice in the 700, 2200, and 7000 ppm (1950; 6129; 19,503 mg/m^3) exposure groups. Following the 26-week recovery period, the incidence of forestomach hyperplasia was greater in the 700 ppm (1950 mg/m^3) and higher exposure groups than in the controls.

Mild to minimal olfactory epithelial degeneration in the nasal cavity was also observed in all mice in the 7000 ppm (19,503 mg/m^3) exposure group after 26 weeks of exposure to isoprene. At the end of the 26-week recovery period, the incidence of mild to moderate olfactory epithelial degeneration was significantly elevated in the 220 ppm (613 mg/m^3) and higher exposure groups.

Exposure-related decreases in testis weight were observed in mice following 26 weeks of exposure to isoprene but not after the recovery period. Testicular atrophy was also observed in male mice exposed to 7000 ppm (19,503 mg/m^3) isoprene for 26 weeks but this effect was not observed after the recovery period.

Lastly, minimal degeneration of the spinal cord white matter was evident in mice exposed to 7000 ppm (19,503 mg/m^3) isoprene for 26 weeks; however, after the 26-week recovery period, the incidence of spinal cord degeneration was significantly increased in all exposure groups. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above.

In the chronic oncogenicity study conducted in B6C3F1 mice, no non-neoplastic lesions were observed. There were no apparent effects on motor function and no exposure-related lesions in the spinal cord at any concentration. This is in sharp contrast to what was observed in the NTP

subchronic study where partial hindlimb paralysis and spinal cord degeneration was observed in mice exposed to 70 ppm (195 mg/m^3) for 6 months.

In the chronic oncogenicity study conducted in rats, non-neoplastic findings included renal tubule hyperplasia and splenic fibrosis. The incidence of renal tubule hyperplasia was significantly greater in males exposed to 7000 ppm (19,503 mg/m^3) isoprene than in the chamber controls. In addition, the severity of kidney nephropathy was slightly increased in 7000 ppm (19,503 mg/m^3) males when compared to chamber controls. The incidences of splenic fibrosis in 700 ppm (1950 mg/m^3) and 7000 ppm (19,503 mg/m^3) males were significantly greater than that in the chamber control group.

Isoprene was tested for mutagenicity in a series of in vivo and in vitro studies. Isoprene was clearly genotoxic to mouse bone marrow in vivo. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and bone marrow micronuclei. However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats. These studies also demonstrate a clear species difference between mice and rats in susceptibility to isoprene. Isoprene was not genotoxic in any of the in vitro assays conducted.

Two-year inhalation carcinogenicity studies were conducted with isoprene in B6C3F1 mice and F344 rats. There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, there were no significant increases in the incidence of malignant tumors. Isoprene exposures in rats were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types makes it difficult to determine if they are truly exposure related. Based on the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene "as reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens and IARC has classified it as Group 2B, possibly carcinogenic to humans.

Isoprene did not produce any maternal or developmental toxicity in rats following exposure to concentrations as high as 7000 ppm (19,503 mg/m^3). However, both maternal and developmental toxicity were evident in mice. In mice, both maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm (19,503 mg/m^3)). Significant reductions in fetal bodyweights were observed at the 280 ppm (780 mg/m^3) dose level for female fetuses and at the 1400 ppm (3900 mg/m^3) level for male fetuses. Thus, in this study, 1400 ppm (3900 mg/m^3) was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/m^3). Again, these studies clearly demonstrate that there is a species difference in sensitivity to isoprene between rats and mice.

In less robust reproductive toxicity evaluations, conducted as part of an NTP subchronic inhalation toxicity study, no significant effects were observed after histopathological evaluations of reproductive organs in rats except slight changes in the testis at the highest exposure level (7000 ppm (19,503 mg/m^3). However, significant effects were observed in mice exposed to isoprene concentrations of 700 ppm (1950 mg/m^3) and higher, including increased estrous cycle length and testicular atrophy as well as decreased epididymal weight. In addition, decreased sperm head count, sperm concentration, and sperm motility were also observed.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Isoprene is expected to exhibit a moderate order of aquatic toxicity based on measured acute effects data that range from approximately 6 to 15 mg/L (Table 5).

Acute Toxicity Test Results

The measured isoprene freshwater fish (*Oncorhynchus mykiss*) 96-hour LC50 is 7.4 mg/L and invertebrate (*Daphnia magna*) 48-hour LC50 is 5.8 mg/L (Huntingdon Life Sciences Ltd., 2003b,c). The measured alga (*Pseudokirchneriella subcapitata*) 72-hour EC50 is 15 and >35 mg/L based on biomass and growth rate, respectively, while the 96-hour EC50 is 16 and >35 mg/L based on biomass and growth rate, respectively (Huntingdon Life Sciences Ltd., 2003d).

Chronic Toxicity Test Results

The measured alga (*Pseudokirchneriella subcapitata*) 72- and 96-hour NOEC for biomass is 1.7 mg/L, while the 72- and 96-hour NOEC for growth rate is 6.0 mg/L (Huntingdon Life Sciences Ltd., 2003d). Measured fish and invertebrate chronic data are not available.

Table 5. Aquatic toxicity data for isoprene

Endpoint	Result (mg/L)
(*Oncorhynchus mykiss*) 96-hour LC_{50}	7.4
(*Daphnia magna*) 48-hour LC_{50}	5.8
(*Pseudokirchneriella subcapitata*) 72- and 96-hour EbC_{50}	16
(*Pseudokirchneriella subcapitata*) 72- and 96-hour ErC_{50}	35
(*Pseudokirchneriella subcapitata*) 72- and 96-hour NOECb	1.7
(*Pseudokirchneriella subcapitata*) 72- and 96-hour NOECr	6.0

b biomassr growth rate

4.2 Terrestrial Effects

There are no experimental data available using standard testing procedures that can be used to assess the terrestrial hazard of isoprene. However, there is a calculated earthworm 14-day LC_{50} value of 311 mg/kg soil (EPIWIN/ECOSAR, 1999). This value was calculated using a log K_{ow} = 2.42.

4.3 Initial Assessment for the Environment

In the air, isoprene has the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with a calculated degradation half-life of 1.2 hours depending on hydroxyl radical concentration. Aqueous photolysis and hydrolysis will not contribute to the transformation of isoprene in aquatic environments because it is either poorly or not susceptible to these reactions.

The photochemical ozone creation potential index for isoprene has been reported to range from 109.2 to 117.8, in comparison with a POCP index of 100 for ethylene, the reference substance.

Because of the relatively short half-life of isoprene in the atmosphere, its contribution to potential global warming can be considered minor.

Results of Mackay Level I distribution modeling at steady state show that isoprene will partition primarily to the air compartment (99.92%), with a negligible amount partitioning to water (0.06%) and soil (0.02%). Level III modeling indicates that at steady state, water is the primary compartment on a percentage basis when a default emission to this compartment is included in the calculations. Level III modeling may not be representative of the ultimate disposition of isoprene because default emission data (1000 kg/h/compartment) used in the model is not a representative rate of chemical discharge. However, concentrations in water are most likely very low because isoprene is quite volatile, and any volatilized substance will be quickly degraded in the atmosphere. When released primarily to the air compartment, the primary mode of removal would be via photodegradation. In spite of its water solubility, wet deposition of isoprene is not likely to play a significant role in its atmospheric fate because of rapid photodegradation. Volatilization to the air will contribute to the rapid loss of isoprene from aqueous and terrestrial habitats.

Isoprene has the potential to biodegrade to a significant extent based on results of ready biodegradation testing. However, microbial metabolism may not greatly contribute to its removal from the environment because of its potential to rapidly volatilize from aquatic and terrestrial media. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate.

Acute aquatic toxicity values for a fish and invertebrate are 7.4 (96hr-LC_{50}) and 5.8 (48hr-EC_{50}) mg/L, respectively. For algae, the lowest 96-hr effect value is 15 mg/L for biomass. Alga 96-hour NOEC values are 1.7 and 6.0 mg/L for biomass and growth rate, respectively.

There are no experimental terrestrial toxicity data available. However, a 14-day LC_{50} value of 311 mg/kg soil has been calculated for an earthworm.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

The chemical possesses properties indicating a hazard for human health (irritation, genotoxic, reproductive and developmental toxicity, carcinogenic) and the environment (fish, invertebrates, algae). Based on data presented by the Sponsor country, relating to production in one country which accounts for approximately 40% of global production and relating to the use pattern in one country, under normal manufacturing, formulation, industrial and consumer use of polymerized isoprene containing products, this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

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rorder=500&rprox=500&rdfreq=500&rwfreq=500&rlead=500&sufs=0&order=r&cq=&id=42377d667.

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S I D S

Dossier

Existing Chemical	:	ID: 78-79-5
CAS No.	:	78-79-5
EINECS Name	:	Isoprene
EC No.	:	201-143-3
TSCA Name	:	1,3-Butadiene, 2-methyl-
Molecular Formula	:	C5H8

Producer related part

Company	:	ExxonMobil Biomedical Sciences Inc.
Creation date	:	26.03.2003

Substance related part

Company	:	ExxonMobil Biomedical Sciences Inc.
Creation date	:	26.03.2003
Status	:	
Memo	:	American Chemistry Council Olefins Panel for the ICCA HPV initiative
Printing date	:	29.07.2005
Revision date	:	
Date of last update	:	29.07.2005
Number of pages	:	
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : Organic
Physical status : Liquid
Purity : ca. 99 % w/w
Colour :
Odour :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2-methyl-1,3-butadiene

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

2-Methylbutadiene

23.02.2004

3-Methyl-1,3-Butadiene

23.02.2004

Beta-Methylbivinyl

23.02.2004

Hemiterpene

23.02.2004

Isoprene

23.02.2004

Methylbivinyl

23.02.2004

1.3 IMPURITIES

Purity	:	
CAS-No	:	138-86-3
EC-No	:	205-341-0
EINECS-Name	:	isoprene dimer (dipentene)
Molecular formula	:	
Value	:	<= .5 % w/w
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

08.08.1997

1.4 ADDITIVES

Purity type	:	
CAS-No	:	98-29-3
EC-No	:	202-653-9
EINECS-Name	:	p-tert.-butyl catechol
Molecular formula	:	
Value	:	
Function of additive	:	
Remark	:	Isoprene contains 150-250 ppm p-tert. butyl catechol as inhibitor/stabilizer.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

08.08.1997

1.5 TOTAL QUANTITY

Remark	:	Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture (SRI International, 2000). In the United States, isoprene production in 1995 was approximately 619 million lb (281,000 Mg [metric tons]) (USITC, 1995).

29.07.2005 (71) (75)

1.6.1 LABELLING

Symbols	:	F+, , ,
Nota	:	, ,

R-Phrases : (12) Extremely flammable
(45) May cause cancer
(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

S-Phrases : (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
(53) Avoid exposure - obtain special instructions before use
(61) Avoid release to the environment. Refer to special instructions/Safety data sets

Remark : 45 -12 - 68 - 52/53
29.07.2005

1.6.2 CLASSIFICATION

Classified :
Class of danger :
R-Phrases : (12) Extremely flammable
(45) May cause cancer
(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits :

Remark : F+; 12 - (Carc. Cat. 2) - 45 - (Muta. Cat. 3) - 68 - 52/53
29.07.2005

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : Industrial
Category : Polymers industry

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
11.01.2005

Type of use : Industrial
Category : Polymers industry

Remark : Isoprene is a chemical intermediate that is used in the synthesis of elastomers, such as poly(cis-1,4-isoprene), butyl-rubber, and coblock polymers.
11.01.2005

Type of use : Industrial
Category : Polymers industry

Remark : Isoprene is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount

of isoprene is used to manufacture specialty chemicals, including vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners.

11.01.2005

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

Remark : Isoprene is obtained by extractive distillation from an isoprene concentrate stream produced by the ethylene production process. In the pyrolysis furnaces of the ethylene production process, paraffinic feedstocks such as ethane, propane, naphthas or gas oils, are subjected to high temperatures in the presence of steam. These conditions result in the partial conversion or cracking of the hydrocarbon feedstock components and formation of unsaturated hydrocarbons. Ethylene and propylene are the primary products, but other olefins, diolefins, aromatics and cyclics are also produced, including a relatively small amount of isoprene. The ethylene process compresses and separates the pyrolysis furnace effluent into product streams. Isoprene produced in the cracking furnace is contained in one of these product streams, the pyrolysis gasoline.

Pyrolysis gasoline is a complex hydrocarbon mixture, consisting predominately of carbon number five (C5+) and higher hydrocarbon components. Distillation of pyrolysis gasoline produces a pyrolysis C5 stream. This stream is also a complex mixture, and consists predominately of the carbon number 5 olefins and diolefins that were produced in the ethylene process cracking furnaces. The stream also includes n-pentane and iso-pentane, which may result largely due to the unconverted pentanes in the ethylene process feedstock. Further processing of the C5 stream results in an isoprene concentrate stream that is separated from the Pyrolysis C5 by a series of distillation and "heat soak" operations. The "heat soak" is used to convert cyclopentadiene to its dimer (dicyclopentadiene) in order to facilitate isolation of the isoprene concentrate. Isoprene concentrate thus produced from the pyrolysis C5 stream has a typical isoprene content of 40%. This concentrate is then processed in an extractive distillation unit that uses a solvent such as acetonitrile to facilitate isolation of the contained isoprene as a 99% purity product.

Isolation of isoprene from the ethylene process co product streams as described above is the primary source of isoprene. "Only this method of production is practiced in the United States and Western Europe. On-purpose synthetic routes to isoprene are also used commercially, including dehydrogenation of isoamylene and isopentane (capacity in Russia) and reaction of isobutylene with formaldehyde (Russia and Japan)."

Source : American Chemistry Council, Olefins Panel

29.07.2005 (40)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Remark : none established
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Remark : Exposure during polymer processing very low under good industrial hygiene and safety at work conditions.

Exposure caused by migration of residue monomer regarded as negligible.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

Remark : Exposure to isoprene may occur at workplaces where it is manufactured. Based on physical properties, the primary workplace exposure would be by inhalation. No consumer exposure is foreseen because there are no direct sales to consumers.

23.02.2004

Remark : Isoprene is a petrochemical that is used as a chemical intermediate in contained systems. Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used.

23.02.2004

Remark : The National Institute for Occupational Safety and Health (NIOSH)

estimated that 3,654 workers (578 of these are female) were potentially exposed to isoprene in the US.

Source : National Institute for Occupational Saftety and Health (NIOSH) (1989). National Occupational Exposure Survey. NIOSH, Washington, DC, USA.

23.02.2004

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered :
Date of search : 04.05.2004

Remark : Aquire (1992 - present)
Biodegradation Data (BIODEG) (1992 - present)
Biodegradation Bibliographic References (BIOLOG)(1992 - present)
Biological Abstracts - BIOSIS (1969 - present)
Cancerlit (1975 - present)
EMBASE (1974 - present)
Enviroline (1970 - present)
Environmental Bibliography (1974 - present)
Gene-Tox (1992 - present)
Medline (1960 - present)
National Technical Information Service (NTIS)(1964 - present)
NIOSH (1973 - present)
PASCAL (1973 - present)
Pollution Abstracts (1970 - present)
TERRETOX (1992 - present)
TSCATS (1977 - present)
Toxfile (1965 - present)

29.07.2005

1.13 REVIEWS

Memo : Metabolism

Remark : Isoprene is metabolized in mammals in processes that involve epoxidation by cytochrome P450-dependent monooxygenases to the isomeric mono-epoxides, (1-methylethenyl)-oxirane and 2-ethenyl-2-methyloxirane Further metabolism of the mono-epoxides to mutagenic isoprene di-epoxides can also occur. The oxidations to the mono- and di-epoxides occur enantioselectively and diastereoselectively. The mono- epoxides are hydrolyzed enantioselectively to vicinal diols under catalysis by epoxide hydrolase. 2-Ethenyl-2-methyloxirane is also readily hydrolyzed non-enzymatically. Because of the stereochemical possibilities for metabolites, the metabolism of isoprene is complex. The metabolism of isoprene by liver microsomes in vitro from a range of species including rat, mouse, and human shows significant differences between species, strains and gender in respect of the diastereoselectivity and enantioselectivity of the metabolic oxidation and hydrolysis reactions. The impact of the extra methyl in isoprene on di-epoxide reactivity also appears to be critically important for the resulting biological effects. Isoprene di-epoxides may exhibit a lower cross-linking potential in vivo compared to butadiene di-epoxides. Differences in metabolism and reactivity of metabolites may be factors

contributing to the significant differences in toxicological response to isoprene observed between species.

29.07.2005 (76)

Memo : Reproduction

Remark : This paper provides a comprehensive review of the genetic toxicology and the reproductive/developmental effects of butadiene and isoprene.

29.07.2005 (1)

2.1 MELTING POINT

Value	:	= -145.9 °C
Sublimation	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	other TS: Isoprene
Test substance	:	Isoprene purity is unknown.
Reliability	:	(2) valid with restrictions The Merck Index is a chemical handbook that is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
Flag	:	Critical study for SIDS endpoint

08.04.2003 (57)

Value	:	= -118.9 °C
Sublimation	:	
Method	:	other: calculated
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Method	:	The calculated value was determined using MPBPWIN version 1.40, a subroutine within the computer program EPIWIN version 3.04. Melting Point estimations performed by MPBPWIN are based on the average result of the calculation methods of K. Joback and Gold and Ogle. Joback's Method is described in Joback, K.G. 1982. A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. In The Properties of Gases and Liquids. Fourth Edition. 1987. R.C. Reid, J.M. Prausnitz and B.E. Poling, Eds. The Gold and Ogle Method simply uses the formula Tm = 0.5839Tb, where Tm is the melting point in Kelvin and Tb is the boiling point in Kelvin.
Reliability	:	(2) valid with restrictions The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.

31.03.2003 (18)

2.2 BOILING POINT

Value	:	= 34 °C at
Decomposition	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	other TS: Isoprene
Test substance	:	Isoprene purity is unknown.
Reliability	:	(2) valid with restrictions The Merck Index is a chemical handbook that is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
Flag	:	Critical study for SIDS endpoint

31.03.2003 (57)

Value : = 35 °C at
Decomposition :
Method : other: calculated
Year :
GLP :
Test substance : other TS: Isoprene

Method : The calculated value was determined using MPBPWIN version 1.40, a subroutine within the computer program EPIWIN version 3.04.
Boiling Point estimations performed by MPBPWIN are based on the calculation method of S. Stein and R. Brown in "Estimation of Normal Boiling Points from Group Contributions". 1994. J. Chem. Inf. Comput. Sci. 34: 581-587.
Reliability : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
31.03.2003 (18)

2.3 DENSITY

Type : density
Value : = .681 g/cm³ at 20 °C
Method :
Year :
GLP : no data
Test substance : other TS: Isoprene

Test substance : Isoprene purity is unknown.
Reliability : (2) valid with restrictions
The Merk Index, an encyclopedia of chemicals, drugs, and biologicals, is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
Flag : Critical study for SIDS endpoint
29.07.2005 (57)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = 733.3 hPa at 25 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : other TS: Isoprene

Method : Method not specified.
Test substance : Isoprene purity is unknown.
Reliability : (2) valid with restrictions
The Handbook of Vapor Pressures and Heats of Vaporization of Hydrocarbons and Related Compounds is a peer reviewed publication.

This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.

Flag : Critical study for SIDS endpoint
31.03.2003 (79)

Value : = 734.6 hPa at 25 °C
Decomposition :
Method : other (calculated)
Year :
GLP :
Test substance : other TS: Isoprene

Method : Calculated values using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.04
Vapor Pressure estimations performed by MPBPWIN are based on the average result of the calculation methods of Antoine and Grain. Both methods use boiling point for the calculation.
The Antoine Method is described in the Handbook of Chemical Property Estimation. Chapter 14. W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, Eds. Washington, D.C.: American Chemical Society. 1990.
A modified Grain Method is described on page 31 of Neely and Blau's Environmental Exposure from Chemicals, Volume 1, CRC Press. 1985.
Reliability : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
31.03.2003 (18)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 2.42 at °C
pH value :
Method :
Year :
GLP : no data
Test substance : other TS: Isoprene

Test substance : Isoprene purity is unknown.
Reliability : (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
Flag : Critical study for SIDS endpoint
29.07.2005 (9)

Partition coefficient :
Log pow : = 2.42 at °C
pH value :
Method :
Year :
GLP : no data
Test substance : other TS: Isoprene

Method : Method not specified.
Test substance : Isoprene purity is unknown.
Reliability : (2) valid with restrictions

The value is cited in the EPIWIN experimental database (SRC Physprop Database) for isoprene. Although the original reference was not retrieved and reviewed for quality, this robust summary has a reliability rating of 2 because the data are from a peer reviewed database.

29.07.2005 (18)

Partition coefficient	:	
Log pow	:	= 2.58 at °C
pH value	:	
Method	:	other (calculated)
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene

Method : Calculated values using KOWWIN version 1.65, a subroutine of the computer program EPIWIN version 3.04
Octanol / Water Partition Coefficient estimations performed by KOWWIN are based on an atom/fragment contribution method of W. Meylan and P. Howard in "Atom/fragment contribution method for estimating octanol-water partition coefficients". 1995. J. Pharm. Sci. 84:83-92.

Reliability : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.

29.07.2005 (18)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 642 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: measured
Year	:	
GLP	:	no
Test substance	:	other TS: Isoprene

Method : From 10 to 20 ml of test substance was added to 200 ml of distilled water and mixed and allowed to settle at 25 °C +/- 1.5 °C. Aqueous samples for analysis were removed from below the organic phase.The aqueous phase was examined for emulsions using phase contrast microscope with a magnification of 1700x and no emulsions were found.

Analysis was by gas chromatograph (GC) with a hydrogen-flame ionization detecter (Beckman). The chromatographic column was 12 ft. x 0.25 in., stainless steel tubing packed with 25% SE 30 gum rubber on 30-60 mesh firebrick. Helium flow through the column was 65cc/min.

Test substance : Isoprene

Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the test procedure and means of analysis suggest that the methodology was appropriate to evaluate the water solubility of a gaseous substance. There is otherwise no

information in the article to suggest that the data are invalid.

Flag : Critical study for SIDS endpoint
29.07.2005 (47)

Solubility in : Water
Value : = 642 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not specified
Year :
GLP : no data
Test substance : other TS: Isoprene

Test substance : Isoprene purity is unknown.
Reliability : (2) valid with restrictions
The value is cited in the EPIWIN experimental database (SRC Physprop Database) for isoprene. Although the original reference was not retrieved and reviewed for quality, this robust summary has a reliability rating of 2 because the data are from a peer reviewed database.
29.07.2005 (18)

Solubility in : Water
Value : = 353 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: calculated
Year :
GLP :
Test substance : other TS: Isoprene

Method : Water solubility calculated by WSKOWWIN, a subroutine of the computer program EPIWIN version 3.11. that is based on a Kow correlation method described by W. Meylan, P. Howard and R. Boethling in "Improved method for estimating water solubility from octanol/water partition coefficient". Environ. Toxicol. Chem. 15:100-106. 1996.
Reliability : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
29.07.2005 (19)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : = -48 °C
Type : closed cup

Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
04.04.2003 (30)

Value : = -54 °C
Type : closed cup
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
27.03.2003

2.8 AUTO FLAMMABILITY

Value : = 427 °C at

Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
04.04.2003 (30)

Value : = 220 °C at
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
27.03.2003

2.9 FLAMMABILITY

Result : extremely flammable
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not

retrieved and reviewed for quality.

04.04.2003 (5) (30)

2.10 EXPLOSIVE PROPERTIES

Result	:	other: forms explosive mixtures with air
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Remark	:	Explosion limits in air: lower limit 1 % vol/vol upper limit 9.7 % vol/vol
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.

27.03.2003

2.11 OXIDIZING PROPERTIES

Result	:	no oxidizing properties
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.

27.03.2003 (5)

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

Memo	:	Isoprene is an unstable, oxidizable liquid.
Remark	:	Isoprene is highly reactive and unless inhibited undergoes explosive polymerization. Polymerization is accelerated by heat and by oxygen and even by the presence of rusty iron. Iron surfaces should be treated with a suitable reducing agent, such as sodium nitrite, before they are placed into isoprene service. When heated to decomposition, isoprene emits acrid smoke and fumes.
Source	:	Exxon Chemical Europe Inc. Bruxelles

27.03.2003 (5)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	at 25 °C
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	= .00000000010514 cm³/(molecule*sec)
Degradation	:	= 50 % after 1.2 hour(s)
Deg. product	:	
Method	:	other (calculated): Calculated values using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Method	:	Calculated values using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions: Temperature: 25°C Sensitizer: OH- radical Concentration of Sensitizer: 1.5E6 OH- radicals/cm3
Remark	:	An approach to assessing the potential for a substance to undergo direct photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by constituent molecules (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, isoprene is not subject to direct photolysis and this degradative mechanism will not contribute to its loss from the environment.
Reliability	:	(2) valid with restrictions The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
Flag	:	Critical study for SIDS endpoint
29.07.2005		(18)
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Method	:	Technical discussion
Remark	:	An approach to assessing the potential for a substance to undergo direct photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by constituent molecules (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, isoprene is not subject to direct photolysis and this degradative mechanism will not contribute to its loss from the environment.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
29.07.2005		(78)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Remark	:	Undergoes photooxidation in the atmosphere and is decomposed to CO and CO2 (Hanst et al., 1980). Reacts with ozone in air yielding formaldehyde (85 %), methacrolein and methylvinylketone (Niki et al., 1983).
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.

29.07.2005 (28) (56)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Isoprene
Result	:	Hydrolysis of an organic molecule can occur when a molecule (R-X) reacts with water (H2O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved. Mechanistically, this reaction is referred to as a nucleophilic substitution reaction, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule. The leaving group, X, must be a molecule other than carbon because for hydrolysis to occur, the R-X bond cannot be a carbon-carbon bond. This reaction differs from other reactions with water such as hydration of carbonyls that can lead to the formation of an alcohol beginning with the transfer of a proton from the water to an alkene. However, water by itself is too weak an acid to transfer a proton in the absence of a strong acid, which could effect such an acid catalysed electrophilic addition. Thus, hydrocarbons such as alkenes are not subject to hydrolysis under conditions typically found within the environment and therefore, this fate process will not contribute to the degradative loss of 2-methyl-2-butene from the environment.
Flag	:	Critical study for SIDS endpoint

07.01.2005 (25) (29)

Remark	:	Isoprene is highly volatile and has a low water solubility. Its specific gravity is less than that of water, therefore it will float on the water (in case of a spill) and is expected to rapidly evaporate from the surface.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

31.03.2003

3.1.3 STABILITY IN SOIL

Deg. product :
Method :
Year :
GLP :
Test substance : other TS: Isoprene

Remark : Based on scientific judgement that considered estimated aerobic biodegradation half-life values, the biodegradation half-life of isoprene in soil is estimated to range from 7 to 28 days.
Reliability : (4) not assignable
This robust summary has a reliability rating of 4 because the data are an estimate based on scientific judgement.
29.07.2005 (31)

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type :
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Henry's Law constant calculation
Year :

Result : The Henry's Law constant (HLC) representing volatility for isoprene is 7,781 Pa-m3/mole at 25°C. The HLC was calculated using a water solubility of 642 mg/L, a vapour pressure of 733.3 hPa, and a molecular weight of 68.12. The vapor pressure and water solubility values are measured values and were obtained from the Syracuse Research Corp. physprop database (EPIWIN).
Test substance : Isoprene
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.
Flag : Critical study for SIDS endpoint
29.07.2005 (18)

Type :
Media : other: air - biota - sediment(s) - soil - water
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Calculation according Mackay, Level I
Year :

Remark : Physicochemical data used in the calculation:

Parameter	Value w/ Units
Molecular Weight	68.12
Temperature	25° C
Log Kow	2.42
Water Solubility	642 g/m3
Vapor Pressure	73330 Pa
Melting Point	-145.9°C

Result : Using the Mackay Level I calculation, the following distribution is predicted for isoprene:

%Distribution	Compartment
99.92	Air
0.06	Water
0.02	Soil
0.00	Sediment
0.00	Suspended Sediment
0.00	Biota

Test substance : Isoprene
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
Flag : Critical study for SIDS endpoint
29.07.2005 (42)

Type :
Media : other: air - biota - sediment(s) - soil - water
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Calculation according Mackay, Level III
Year :

Remark : Physicochemical data used in the calculation:

Parameter	Value w/ Units
Molecular Weight	68.12
Temperature	25° C
Log Kow	2.42
Water Solubility	642 g/m3
Vapor Pressure	73330 Pa
Melting Point	-145.9°C

Emissions rates used in the calculation:

Compartment	Rate (kg/hr)
Air	1000
Water	1000
Soil	1000

Half-lives used in the calculation:

Compartment	Half-life (hr)

Air	1.2a
Water	120b
Soil	420c
Sediment	420c

a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.]
b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.

Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA.
c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment.

Result : Using the Mackay Level I calculation, the following distribution is predicted for isoprene:

Compartment	%Distribution
Air	3.11
Water	87.72
Soil	8.96
Sediment	0.21

Test substance : Isoprene
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
Flag : Critical study for SIDS endpoint
29.07.2005 (43)

Type :
Media : other: air - biota - sediment(s) - soil - water
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Calculation according Mackay, Level III
Year :

Remark : Physicochemical data used in the calculation:

Parameter	Value w/ Units
Molecular Weight	68.12
Temperature	25° C
Log Kow	2.42
Water Solubility	642 g/m3
Vapor Pressure	73330 Pa
Melting Point	-145.9°C

Emissions rates used in the calculation:

Compartment	Rate (kg/hr)
Air	1000
Water	0
Soil	0

Half-lives used in the calculation:

Compartment	Half-life (hr)
Air	1.2a
Water	120b
Soil	420c
Sediment	420c

a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.]
b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.

Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA.
c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment.

Result : Using the Mackay Level III calculation, the following distribution is predicted for isoprene:

Compartment	%Distribution
Air	99.96
Water	0.02
Soil	0.02
Sediment	0.00

Test substance : Isoprene
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
Flag : Critical study for SIDS endpoint
29.07.2005 (43)

Type :
Media : other: air - biota - sediment(s) - soil - water
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Calculation according Mackay, Level III
Year :

Remark : Physicochemical data used in the calculation:

Parameter	Value w/ Units

		Molecular Weight 68.12 Temperature 25° C Log Kow 2.42 Water Solubility 642 g/m3 Vapor Pressure 73330 Pa Melting Point -145.9°C Emissions rates used in the calculation: Compartment Rate (kg/hr) Air 0 Water 1000 Soil 0 Half-lives used in the calculation: Compartment Half-life (hr) Air 1.2a Water 120b Soil 420c Sediment 420c a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.] b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A. Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA. c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment.
Result	:	Using the Mackay Level I calculation, the following distribution is predicted for isoprene: Compartment %Distribution Air 0.42 Water 99.34 Soil 0.00 Sediment 0.24
Test substance	:	Isoprene
Reliability	:	(2) valid with restrictions This robust summary has a reliability rating of 2 because the data are calculated.
Flag	:	Critical study for SIDS endpoint
29.07.2005		(43)

3.3.2 DISTRIBUTION

Media :
Method : other (calculation)
Year :

Method : The calculated value was determined using PCKOCWIN version 1.66, a subroutine within the computer program EPIWIN version 3.04.
Result : Koc = 67.7
Log Koc = 1.83
Test substance : Isoprene
Reliability : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are not measured.
Flag : Critical study for SIDS endpoint
29.07.2005 (18)

3.4 MODE OF DEGRADATION IN ACTUAL USE

Memo : Main route of isoprene degradation is through atmospheric oxidation by OH radicals. The atmospheric half-life is expected to be 1.2 hours.

Remark : Isoprene is expected to volatilize from soil or open waters rapidly. As isoprene volatilizes, it is subject to oxidation predominantly by hydroxyl radical attack. In the aquatic environment, isoprene is resistant to hydrolysis since it lacks hydrolyzable groups. Photolysis is an unlikely route of degradation for isoprene. Biodegradation is of minor importance since isoprene will not persist in the soil or aquatic environment long enough for a significant amount of biodegradation to occur.
Source : Exxon Chemical Europe Inc. Bruxelles
01.03.2004

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, domestic, non-adapted
Concentration : 51 mg/l related to Test substance
related to
Contact time : 28 day(s)
Degradation : = 61 (±) % after 28 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 10 day(s) = 10 %
18 day(s) = 60 %
28 day(s) = 61 %
%
%
Control substance : Benzoic acid, sodium salt
Kinetic : 28 day(s) = 93 %
%
Deg. product :
Method : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year : 2004
GLP : yes
Test substance : other TS: Isoprene (CAS No. 78-79-5)
Result : The biodegradation half-life <2 weeks. By day 28, 60.9% degradation of

the test material was observed. 10% biodegradation was achieved on approximately day 10, 50% biodegradation on approximately day 13, and >60% biodegradation on day 18. However, isoprene was not readily biodegradable because the replicate data exceeded the allowable range (53 to 75%).

By day 2, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted.

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
Isoprene	53.4, 54.6, 74.8	60.9
Na Benzoate	89, 91, 100	93

* replicate data

Test condition : Activated sludge and test medium were combined per OECD Guideline 301F prior to test material addition. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) per OECD Guideline 301F.

Test vessels were 1L glass flasks placed in a waterbath and electronically monitored for oxygen consumption. Test material was tested in triplicate, controls and blanks were tested in duplicate.

Test material (isoprene) concentration was 51 mg/L. The positive control (sodium benzoate) concentration was 46 mg/L. Test temperature was 22 +/- 1 Deg C.

All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Test substance : Isoprene (CAS No. 78-79-5)
Purity: >99%

Conclusion : The test material is not readily biodegradable, but exhibited a high extent of biodegradation.

Reliability : (2) valid with restrictions
This summary represents a key study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and the results were reviewed for reliability and assessed as valid. The study was given a reliability of 2 because the replicate data exceded the allowable range.

Flag : Critical study for SIDS endpoint

29.07.2005 (20)

Type : aerobic
Inoculum : other: domestic sewage effluent
Concentration : 2 mg/l related to Test substance
related to
Contact time : 28 day(s)
Degradation : (±) % after
Result :
Deg. product :
Method : other:
Year : 2002
GLP : yes
Test substance : other TS: Isoprene

Method : OECD 301D; US EPA OPPTS 835.3110; and EC Directive 92/69, C.4-E

Result : The mean Total Viable Count of microorganisms in the sample of final sewage effluent in the main test was 7.1 x 105 colony forming units (cfu) per ml and the mean count in inoculated mineral salts medium on day 0 was 3.3 x 104 cfu/ml.

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

A maximum extent of 60% biodegradation was measured in one test system on day 18 of the main Closed Bottle test. On day 28, the average biodegradation was 30% (2 and 58%). A maximum extent of 64% biodegradation was measured on day 7 of the supplementary investigation, which confirms that isoprene can be rapidly biodegraded in the presence of an acclimated inoculum. The test substance showed no inhibitory effect on the normal degradative activity of the microbial inoculum in the supplementary study.

	% Degradation*		
Sample	Day 5	Day 18	Day 28
Test material	5, 2	2, 60	2, 58
Na Benzoate	78, 79	---	86, 86

* replicate data from non acclimated test systems

Test condition : A sample of secondary effluent was collected on the day of the test from a trickling-filter plant and maintained under aerobic conditions in the laboratory. Immediately before use, it was filtered through glass wool and the filtrate used as the inoculum for the test. Eighteen test system bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/L, and the test substance at a nominal loading of 2 mg/l.

Two additional series of eighteen bottles each were filled with inoculated mineral salts medium. The reference substance, sodium benzoate, at a nominal loading of 5 mg/l was added to one series, while the second series was used to determine background respiration. DO (dissolved oxygen) concentrations in replicate bottles from each series of eighteen bottles were initially measured on day 0 and following incubation at 22 ± 2°C in darkness on days 5, 7, 11, 14, 18, 21, 25, and 28. An additional four bottles, each containing inoculated mineral salts medium with test and reference substances, were used to assess the impact of the test substance on the degradative activity of the the inoculum. DO concentrations were determined on days 0 and 5.

In a supplementary investigation, eight bottles were established with mineral salts medium and an inoculum obtained from a BOD bottle in which isoprene biodegradation had achieved 58% on day 28. Four bottles contained isoprene, while the second four bottles acted as controls containing inoculated medium alone. The DO, pH, and temperature of two bottles containing isoprene and two bottles containing inoculum and mineral salts medium alone were analysed on day 0. The remaining bottles were incubated for a total of 7 days in darkness at 22 ± 2°C and then analysed.

Test substance : Purity: 98.6% w/w (analysis of 16/11/01)
98.5% w/w (analysis of 23/7/02)
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry well-ventilated area in the dark under

nitrogen
Supplier: Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicester, LE11 5RG, USA
Date received by lab conducting test: 16 May 2001

The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (89%) was equivalent to 101% of the theoretical value (88.16%).

Conclusion : Isoprene has the potential to biodegrade to a significant extent under non acclimated and acclimated test conditions.

The results of selected individual replicate test systems from this study for sampling days 18 through 28 are outside guideline requirements, which brings the validity of the the study into question. However, it is equally questionable that valid replicate data would be achieved in subsequent tests under a similar test design. When the individual test system results are considered, it is clear from this study that an 18-day lag period occurred thorugh the day 14 samples (replicate day 14 results were 5 and 5%). All other test systems prior to day 14 exhibited less than 5% biodegradation. These data suggest that initiating biodegradation may be metabolically challenging. In comparison, results from day 18 through 28 replicate samples range from 54 to 60% for three samples and 2 to 13% for five samples. These data suggest that biodegradation once initiated can proceed to a high extent, but test conditions can limit the opportunity for acclimation with a subsequent high extent of biodegradation to occur.

Reliability : (2) valid with restrictions
This study is assigned a reliability of "2" because the range of replicate results were not within guideline requirements. No other guideline deviations occurred. Although this study was conducted according to guideline methods, the replicate test systems exhibited greater than 20% variability on days 18 through 28 of the study. However, the data from this study can still be used to evaluate the potential biodegradability of the test material with an understanding of the test system limitations and the potential for the type of results exhibited in this study.

01.03.2004 (33)

Type : aerobic
Inoculum :
Concentration : 2 mg/l related to Test substance
related to
Contact time : 28 day(s)
Degradation : = 2 (±) % after 28 day(s)
Result :
Deg. product :
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year :
GLP :
Test substance : other TS: Isoprene (CAS No. 78-79-5)

Result : At test concentrations of both 2 and 10 mg/L, the test material exhibited 2% biodegradation after 28 days.

Test condition : The test was conducted using a guideline that corresponds to the OECD 301C, Modified MITI Test. The test material was evaluated at 2 and 10 mg/L. The inoculum was developed from sludge obtained from 4 sewage plants, 3 rivers, 1 lake, and 2 bays according to methods described in the OECD guideline.

Test substance : Isoprene (CAS No. 78-79-5)

Reliability : (2) valid with restrictions

29.07.2005 (9)

Type	:	aerobic
Inoculum	:	Arthrobacter sp. (Bacteria)
Concentration	:	385 µg/l related to 500 µg/l related to
Method	:	GC/PID analysis of soil core headspace.
Remark	:	Sealed oil cores were used to evaluate isoprene vapor degradation in the field and lab. Alfisol from a temperate mixed-hardwoods forest near Ithaca, NY, US had a pH 5.8-6, 9% organic matter and 57% porosity.
Result	:	Headspace isoprene concentrations fell from approx 385 ppb at time 0 to < 5ppb by 2 hr in the soil cores maintained in the field. During the four month (June - Oct 1996) experiment, headspace samples were collected monthly; after sampling the isoprene was reinjected into the core headspace. Monthly analysis for isoprene showed average degradation rates approx. 2000 pmol/gram soil/day. Soil cores maintained in the lab and dosed with isoprene @ 500 ppm showed complete removal from the headspace after 18 hours.
Source	:	Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(2) valid with restrictions This robust summary has a reliability rating of 2 because the data were developed using non standardized test procedures. However, the information is well documented and meets accepted scientific principles.

29.07.2005 (10)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species	:	other: see remark
Exposure period	:	at °C
Concentration	:	
Elimination	:	
Method	:	OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year	:	
GLP	:	no data
Test substance	:	other TS: Isoprene
Remark	:	The species tested was the carp (Cyuprinus carpio) supplied by Sugishima fish farm, Kumamoto, Japan. All fish were acclimated for approximately 28 days prior to test initiation.
Result	:	Measured log bioconcentration factor (BCF) values were determined as 0.7 to 1.1 (BCF = 5.0 to 14) and 0.7 to 1.3 (BCF = 5.6 to 20) at exposure concentrations of 50 and 5 mg/L, respectively.
Test condition	:	Test water was ground water from Kurume Research Laboratory. Water quality parameters were monitored requlary and included temperature, pH, and disolved oxygen. Additionally, total hardness, COD, and contaminants were analyzed regularly on site. Dilution water met ministerial ordinance of the Ministry of Health and Welfare for total hardness and residue. Test temperature was 25 +/- 2 degree C. Fish weight was approximately 30g, length approximately 10 cm, and lipid content 2 to 6% body weight. Fish were fed twice daily a daily total amount corresponding to

approximately 2% total body weight. Fish were not fed on sampling days. Water flow rate was 200 to 800 ml/min. Dissolved oxygen ranged from 6 to 8 mg/L. Up to 20 fish may have been used per study.

Samples were analyzed as follows:
Test material exposure solution - 1 sample twice a week
Test fish - 2 samples every two weeks
Control fish - 2 samples before test initiation and at termination

Recovery efficiencies were determined and used as correction factors for the determination of test substance concentration in test samples.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
29.07.2005 (9)

Species : other: see remark
Exposure period : at °C
Concentration :
BCF : = 14.57
Elimination :
Method : other: calculation
Year :
GLP : no data
Test substance : other TS: Isoprene

Remark : A log BCF of 1.163 (BCF = 14.57) is calculated. With respect to the log Pow = 2.42, isoprene in the aquatic environment is expected to have a low potential for bioaccumulation.
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.
29.07.2005 (18)

Remark : Could not confirm reported log P, data not in reference, but other log P data supports low BCF potential.
The water solubility of isoprene of 0.38 g/l is such low, that bioaccumulation is not expected to occur, although the log Pow being about 3.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (8)

3.8 ADDITIONAL REMARKS

Remark : The photochemical ozone creation potential (POCP) index for a chemical provides a relative measure of its reactivity or ozone forming potential. The POCP index can also provide a means of ranking volatile organic compounds (VOCs) by their ability to form ozone in the troposphere. Reported POCP indices for isoprene in northwestern Europe range from 109.2 to 117.8, in comparison with an POCP index of 100 for ethylene, the reference substance.
Reliability : (2) valid with restrictions
The values were calculated. This robust summary has a reliability rating of 2 because the data are not measured.
29.07.2005 (16) (17)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 7.43 measured/nominal
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 2002
GLP : yes
Test substance : other TS: Isoprene

Result : 96-hour LC50 = 7.43 mg/L (95% CI = 6.71 and 15.0 mg/l) based upon measured values

Nominal Conc. (mg/L)	Mean Measured Conc. (mg/L)	Fish Total Mortality (@96 hrs)*
Control	Not Detected	0
2.13	1.68	0
4.70	3.57	0
10.3	6.71	4
22.7	15.0	10
50.0	28.7	10

*10 fish total added to control and each treatment at test initiation

Test condition : Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 24 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, aspirator bottles (capacity approximately 11.4 L) with 5 fish in each vessel. The fish were exposed for a period of 96 hours with daily batch renewal of the media. Daily renewals were performed by removing ~80% of the test solution through the port at the bottom and refilling with fresh solution.

Nominal treatment levels were: control, 2.13, 4.70, 10.3, 22.7, and 50.0 mg/L, which measured: not detected, 1.68, 3.57, 6.71, 15.0, and 28.7 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old solutions.

Test temperature was 14.1 to 15.4 Deg C., Lighting was 16 hours light : 8 hours dark with periods of subdued lighting at the beginning and end of each light phase.

Dissolved Oxygen at initiation ranged from 97 to >100% of the air saturation value (ASV) and from 31 to 99% ASV in "old" solutions prior to renewals. The pH ranged from 7.3 to 8.1 during the study. Total hardness was within the range of 160 to 190 mg as CaCO3. Fish were not fed during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

Test substance : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area
Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001
Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

Reliability : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.

Flag : Critical study for SIDS endpoint

03.04.2003 (34)

Type :
Species : Carassius auratus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 180

Remark : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used. Test systems were static, and open, which allowed for volatile loss of test substance.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

Type :
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 43

Remark : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used. Test systems were static, and open, which allowed for volatile loss of test substance.

Source : Deutsche Shell Chemie GmbH Eschborn

Exxon Chemical Europe Inc. Bruxelles

Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

Type :
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 75

Remark : Hard water
LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used.
Test systems were static, and open, which allowed for volatile loss of test substance.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

Type :
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96
Unit : mg/l
LC50 : = 87

Remark : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used.
Soft water
Test systems were static, and open, which allowed for volatile loss of test substance.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 5.77 measured/nominal

Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2002
GLP : Yes
Test substance : other TS: Isoprene

Result : 48-hour EC50 = 5.77 mg/L (95% CI = 3.52 and 9.47 mg/l) based upon measured values

Nominal Conc. (mg/L)	Mean Measured Conc. (mg/L)	Daphnid Immobility (@48 hrs)*
Control	Not Detected	0
2.13	0.65	0
4.70	1.55	0
10.3	3.52	0
22.7	9.47	20
50.0	24.6	20

*20 daphnids total added to control and each treatment at test initiation

Test condition : Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 22 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, glass vessels with 10 daphnids in each vessel. The daphnids were exposed for a period of 48 hours without renewal of the media.

Nominal treatment levels were: control, 2.13, 4.70, 10.3, 22.7, and 50.0 mg/L, which measured: not detected, 0.648, 1.55, 3.52, 9.47, and 24.6 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old (test termination) solutions.

Test temperature was 14.1 to 15.4 Deg C., Lighting was 16 hours light : 8 hours dark with periods of subdued lighting at the beginning and end of each light phase.

Dissolved Oxygen at initiation ranged from 97 to >100% of the air saturation value (ASV) and from 31 to 99% ASV in "old" solutions prior to renewals. The pH ranged from 7.3 to 8.1 during the study. Total hardness was within the range of 160 to 190 mg as CaCO3. Daphnids were not fed during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

Test substance : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area
Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001

Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

Reliability : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.
Flag : Critical study for SIDS endpoint
07.01.2005 (35)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = 260
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.
29.07.2005 (66)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 140
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.
29.07.2005 (66)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : other algae: Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) (Algae)
Endpoint : Biomass
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 1.68 measured/nominal

EC50 : = 15.5 measured/nominal
Limit test :
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 2002
GLP : Yes
Test substance : other TS: Isoprene

Result : Area under the growth curve:
EbC50 (72 h): 15.3 mg/l (95% CI = 12.9 and 18.6 mg/l)
EbC50 (96 h): 15.5 mg/l (95% CI = 13.3 and 18.4 mg/l)
No observed effect concentration (NOEC, 72/96 h): 1.68 mg/l

Average specific growth rate:
ErC50 (72h): >35.2 mg/l (34% inhibition at 35.2 mg/L)
ErC50 (96 h): >35.2 mg/l (31% inhibition at 35.2 mg/L)
No observed effect concentration (NOEC, 72/96 h): 6.00 mg/l

Nominal Conc. (mg/L)	Mean Measured Conc. (mg/L)	Alga Cell Count (cells/ml @96 hrs)
Control	Not Detected	117 E
4.27	0.85	117 E
9.39	1.68	110 E
20.7	6.00	111 E
45.5	10.3	74.9 E
100.0	35.2	28.1 E

Test condition : Treatment solutions were prepared either individually or by dilution of a treatment solution. Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 21 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, glass vessels with an initial cell density of 1 x 104/ml. The algae were exposed for a period of 96 hours without renewal of the media.

Nominal treatment levels were: control, 4.27, 9.39, 20.7, 45.5, and 100 mg/L, which measured: not detected, 0.846, 1.68, 6.00, 10.3, and 35.2 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old (test termination) solutions.

The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.4 to 24.0 Deg C. The pH ranged from 7.3 to 8.1 during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

Test substance : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area

Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001
Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

Reliability : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.
Flag : Critical study for SIDS endpoint
29.07.2005 (36)

Species : other algae: green alga
Endpoint :
Exposure period : 96 hour(s)
Unit : mg/l
ChV* : = 1.83 calculated
Method :
Year :
GLP :
Test substance : other TS: Isoprene

Test condition : A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(C)C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C).
Reliability : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.
01.03.2004 (18) (27)

Species : Scenedesmus quadricauda (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : > 1000
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.
29.07.2005 (67)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

Species	:	other: fish
Endpoint	:	
Exposure period	:	30 day(s)
Unit	:	mg/l
ChV*	:	= 2.81 calculated
Method	:	other: ECOSAR Computer Model (in:EPIWIN)
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Remark	:	Test Type: Chronic Fish Toxicty Calculation
Test condition	:	A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(C)C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C).
Reliability	:	(2) valid with restrictions This robust summary has a reliability rating of 2 because the data are calculated and not measured.

31.03.2003 (18) (27)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	other: Daphnid
Endpoint	:	
Exposure period	:	16 day(s)
Unit	:	mg/l
EC50	:	= 1.38 calculated
Method	:	other: ECOSAR Computer Model (in:EPIWIN)
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Remark	:	Test Type: Chronic Daphnid Toxicity Calculation
Test condition	:	A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(-C)-C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C).
Reliability	:	(2) valid with restrictions This robust summary has a reliability rating of 2 because the data are calculated and not measured.

31.03.2003 (18) (27)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type	:	other: Earthworm Toxicity Calculation
Species	:	other: earthworm
Endpoint	:	Mortality
Exposure period	:	14 day(s)
Unit	:	other: ppm
LC50	:	= 311.11 calculated
Method	:	other: ECOSAR Computer Model (in: EPIWIN)
Year	:	
GLP	:	
Test substance	:	other TS: Isoprene
Test condition	:	A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(-C)-C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C).
Reliability	:	(2) valid with restrictions This robust summary has a reliability rating of 2 because the data are calculated and not measured.

31.03.2003 (18) (27)

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 2043 - 2210 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: isoprene in oil
Remark	:	15 male/15 female Wistar rats were administered single doses of isoprene in oil by stomach tube. LD50 was calculated.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable Insufficient experimental detail to assess quality.

29.07.2005 (38)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	= 180 mg/l
Species	:	rat
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	other: Not applicable
Doses	:	
Exposure time	:	4 hour(s)
Method	:	other
Year	:	1969
GLP	:	
Test substance	:	other TS: Isoprene
Remark	:	No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.
Result	:	Rat LC50 (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p<0.05$). Mouse LC50 (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p<0.05$).
Test condition	:	Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon.
Conclusion	:	LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L

(56,363 ppm) in mice.
Reliability : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.
29.07.2005 (68)

Type : LC50
Value : = 157 mg/l
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : other: Not applicable
Doses :
Exposure time : 2 hour(s)
Method : other
Year : 1969
GLP :
Test substance : other TS: Isoprene

Remark : No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.
Result : Rat LC50 (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p<0.05$).
Mouse LC50 (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p<0.05$).
Test condition : Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon.
Conclusion : LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L (56,363 ppm) in mice.
Reliability : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.
29.07.2005 (69)

Type : LC50
Value : = 157 mg/l
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 2 hour(s)
Method :
Year :
GLP : no
Test substance :

Remark : No data on number and sex of animals and on post observation period.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.
29.07.2005 (44)

Type	:	LC50
Value	:	= 214 mg/l
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	4 hour(s)
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	No data on number and sex of animals and on post observation period.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable Lethality study only; insufficient experimental detail to assess quality.

29.07.2005 (44)

Type	:	LC50
Value	:	= 135 - 153 mg/l
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	2 hour(s)
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	males: LC50 = 135-143 mg/l females: LC50 = 144-153 mg/l No data on number of animals and post observation period.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable Lethality study only; insufficient experimental detail to assess quality.

29.07.2005 (24)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	no

Test substance :

Remark : 5 rats received single application of 1 ml isoprene each on their back skin (animals had been shaved the day before application). The substance was not removed for 7 days. An LD50 of > 1 ml/kg b. w. was established.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (38)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : = 1310 - 1470 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals :
Vehicle :
Doses :
Route of admin. : i.p.
Exposure time :
Method :
Year :
GLP : no data
Test substance :

Remark : Doses from 100 to 1750 mg/kg b. w. were administered.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (38)

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration :
Exposure :
Exposure time :
Number of animals :
Vehicle :
PDII :
Result : slightly irritating
Classification : not irritating
Method :
Year :
GLP : no data
Test substance : other TS: distillate cut containing 50 % isoprene and 50 % C5

Remark : Prolonged contact, slight redness;
repeated contact, slight chemical burn.
Not absorbed in toxic amounts in a 3-day occlusion test.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
Insufficient experimental detail to assess quality.

29.07.2005 (37)

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Remark	:	One ear each of 2 New Zealand rabbits were painted on 5 consecutive days 2 times a day with isoprene. A reversible redness staying for a short while resulted.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable Insufficient experimental detail to assess quality.

29.07.2005 (38)

Species	:	rat
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	Isoprene penetrates the intact skin and causes local irritations.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles
Reliability	:	(4) not assignable Insufficient experimental detail to assess quality.

29.07.2005 (24)

Species	:	mouse
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	Isoprene penetrates the intact skin and causes local

irritations.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
Insufficient experimental detail to assess quality.
29.07.2005 (24)

Species : rabbit
Concentration :
Exposure :
Exposure time :
Number of animals :
Vehicle :
PDII :
Result : slightly irritating
Classification : not irritating
Method :
Year :
GLP : no
Test substance :

Remark : Isoprene penetrates the intact skin and causes local irritations.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
Insufficient experimental detail to assess quality.
29.07.2005 (24)

5.2.2 EYE IRRITATION

Remark : Isoprene caused eye irritation. No further data given.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
Reliability : (4) not assignable
Insufficient experimental detail to assess quality.
29.07.2005 (44)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type :
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : inhalation
Exposure period : 6 hours/day
Frequency of treatm. : 5 days/week for 2 weeks
Post exposure period : not applicable
Doses : 0, 438, 875, 1750, 3500, or 7000 ppm
Control group : yes
NOAEL : = 7000 ppm
LOAEL : > 7000 ppm

Method	:	other
Year	:	1990
GLP	:	yes
Test substance	:	other TS: Isoprene
Method	:	Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test.
Remark	:	Control group and treatment: air-only exposed
Result	:	In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or the incidence of gross or microscopic lesions.
Test condition	:	Groups of 20 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group were used for clinical pathology evaluations after 4 exposures. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	Isoprene exposures over 2 weeks produced no changes in any of the measured parameters in the rat at exposures up to 7000 ppm.
Reliability	:	(1) valid without restriction Comparable to guideline study (OECD 412).

29.07.2005 (48)

Type	:	
Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	inhalation
Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week for 2 weeks
Post exposure period	:	not applicable
Doses	:	0, 438, 875, 1750, 3500, or 7000 ppm
Control group	:	yes
LOAEL	:	= 438 ppm
Method	:	other
Year	:	1990
GLP	:	yes
Test substance	:	other TS: Isoprene
Method	:	Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test.
Remark	:	Control group and treatment: air-only exposed
Result	:	In mice, there were no effects on survival; the mean body weight gain of males in the 7,000 ppm group was less than that of the controls. In mice, exposure to isoprene caused decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in all exposed groups. Organ weight changes were observed in both male and female mice; increased liver weights and decreased thymus, spleen, and testis weights were observed in all exposed groups. Microscopic lesions observed in the exposed mice included atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach.
Test condition	:	Groups of 20 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group were used for clinical

pathology evaluations after 5 exposures. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found.

Test substance : Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion : Isoprene exposures over 2 weeks induced changes in hematological parameters, body and organ weights, and microscopic appearances in certain tissues at levels as low as 438 ppm.
Reliability : (1) valid without restriction
Comparable to guideline study (OECD 412).

29.07.2005 (48)

Type :
Species : Rat
Sex : male/female
Strain : Fischer 344
Route of admin. : Inhalation
Exposure period : 6 hours/day
Frequency of treatm. : 5 days/week for 13 weeks
Post exposure period : not applicable
Doses : 0, 70, 220, 700, 2200, or 7000 ppm
Control group : Yes
NOAEL : = 7000 ppm
LOAEL : > 7000 ppm
Method : other
Year : 1994
GLP : Yes
Test substance : other TS: Isoprene

Method : Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Remark : Control group and treatment: air-only exposed
Result : In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions.
Test condition : Groups of 10 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats were sacrificed and evaluated histopathologically. Organ weights were recorded.
Test substance : Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion : No toxicological effects were evident in rats exposed up to 7000 ppm isoprene for 13 weeks.
Reliability : (1) valid without restriction
Comparable to guideline study (OECD 413).

29.07.2005 (49)

Type :
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : Inhalation

Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week for 13 weeks
Post exposure period	:	not applicable
Doses	:	0, 70, 220, 700, 2200, or 7000 ppm
Control group	:	Yes
NOAEL	:	= 220 ppm
LOAEL	:	= 700 ppm
Method	:	other
Year	:	1994
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Remark	:	Control group and treatment: air-only exposed
Result	:	In mice, there were no effects on survival, body weight gain, or clinical signs. However, male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. The incidences of focal epithelial hyperplasia of the forestomach were 0, 0, 0, 9, 8, 9 in the males, and 0, 0, 0, 10, 9, 10 in the females at 0, 70, 220, 700, 2200, and 7000 ppm (n=10). Degeneration of the olfactory epithelium and cytoplasmic degeneration of the liver were observed in 10/10 male mice at 7000 ppm. The male mice exposed to 7000 ppm exhibited testicular weights reduced 35% compared to the controls.
Test condition	:	Groups of 10 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from mice during week 12. After thirteen weeks of exposures, all mice were sacrificed and evaluated histopathologically. Organ weights were recorded.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	In mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week subchronic inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed the species difference between rats and mice in susceptibility to isoprene.
Reliability	:	(1) valid without restriction Comparable to guideline study (OECD 413).

29.07.2005 (49)

Type	:	
Species	:	Rat
Sex	:	Male
Strain	:	Fischer 344
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week for 26 weeks
Post exposure period	:	26-week post-exposure recovery period
Doses	:	0, 70, 220, 700, 2200, or 7000 ppm
Control group	:	Yes
NOAEL	:	2200 ppm
LOAEL	:	= 7000 ppm
Method	:	other
Year	:	1994
GLP	:	Yes
Test substance	:	other TS: Isoprene

Method	:	Analysis of survival and incidence of nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Remark	:	Control group and treatment: air-only exposed
Result	:	The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis (10/10) in the 7000 ppm group; following the 26-week recovery period the only effect in rats was a marginal increase in benign testicular interstitial cell tumors (9/30 at 7000 ppm).
Test condition	:	Groups of 40 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. At the end of the 26-week exposure period, 10 rats/group were sacrificed and evaluated. The remaining animals were allowed to recovery for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis in the 7000 ppm group. Following the 26 week recovery period a marginal increase in benign testicular interstitial cell tumors was observed in the highest dose group, i.e. 7000 ppm.
Reliability	:	(2) valid with restrictions Comparable to guideline studies. This study involved exposures of male rats to isoprene for 6 months, therefore provided additional data on repeated dose toxicity.

29.07.2005 (49)

Type	:	
Species	:	Mouse
Sex	:	Male
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week for 26 weeks
Post exposure period	:	26-week post-exposure recovery period
Doses	:	0, 70, 220, 700, 2200, or 7000 ppm
Control group	:	Yes
NOAEL	:	= 70 ppm
LOAEL	:	= 700 ppm
Method	:	other
Year	:	1994
GLP	:	Yes
Test substance	:	other TS: Isoprene

Method	:	Analysis of survival and incidence of nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
Remark	:	Control group and treatment: air-only exposed
Result	:	Survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. Non-neoplastic lesions were observed in male mice exposed to isoprene and included spinal cord degeneration (>70 ppm) and degeneration of the olfactory epithelium (>220 ppm). Slight increases in testicular atrophy, epithelial hyperplasia of the forestomach, partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in male mice.

Selected non-neoplastic lesions were as follows (0, 70, 220, 700, 2200, 7000 ppm) -
After 26 weeks exposure:
Nasal turbinates/olfactory epithelial degeneration - 0/10, 0/10, 0/10, 1/10, 1/10, 10/10.
Testes/atrophy - 0/10, 0/10, 0/10, 0/10, 1/10, 5/10.
Spinal cord/degeneration - 0/10, 0/10, 0/10, 0/10, 1/10, 10/10.
After 26 weeks recovery:
Nasal turbinates/olfactory epithelial degeneration - 1/30, 2/30, 5/29, 11/30, 25/30, 28/28.
Testes/atrophy - 0/30, 0/30, 0/29, 0/30, 0/30, 3/29.
Spinal cord/degeneration - 4/30, 20/30, 19/29, 17/29, 13/28.

Test condition : Groups of 40 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. At the end of the 26-week exposure period, 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recovery for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals. Twenty mice/group were evaluated for forelimb and hindlimb grip strength after 26 weeks exposure; 10 mice/group were also evaluated at 2 days, 1-, 3-, and 6-months post-exposure.

Test substance : Isoprene (CAS# 78-79-5) purity > 99%.

Conclusion : Non-neoplastic lesions related to treatment included forestomach squamous-cell hyperplasia, lung alveolar hyperplasia, nasal olefactory degeneration, and spinal cord degeneration.

Reliability : (2) valid with restrictions
Comparable to guideline studies. This study involved exposures of male rats and male mice to isoprene for 6 months, therefore provided additional data on repeated dose toxicity and carcinogenicity.

29.07.2005 (49)

Type :
Species : Rat
Sex : male/female
Strain : Fischer 344
Route of admin. : Inhalation
Exposure period : 6 hours/day
Frequency of treatm. : 5 days/week for 104 weeks
Post exposure period : None
Doses : 0, 220, 700, or 7000 ppm
Control group : Yes
Method : other
Year : 1997
GLP : Yes
Test substance : other TS: Isoprene

Method : Analysis of survival and incidence of nonneoplastic lesions was performed. Urine data was analyzed by nonparametric methods.

Remark : Control group treatment: 50 male and 50 female rats exposed to air only

Result : Survival of all exposed groups was similar to the chamber controls. There were no exposure-related changes in clinical observations or body weights. The incidences of splenic fibrosis in the 700 and 7,000 ppm males (24/50, 22/50) were significantly greater than that in the chamber control group (11/50).

Test condition : Groups of 50 rats/sex/group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104

weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed.

Test substance : Isoprene (CAS# 78-79-5) purity > 99.7%.

Conclusion : In this study survival of all exposed groups were similar to the chamber controls. There were no exposure related changes in clinical observations or body weights.

Reliability : (1) valid without restriction

11.01.2005 (54)

Type :
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : Inhalation
Exposure period : 4 or 8 hours/day
Frequency of treatm. : variable - 5 days/week for 20, 40 or 80 weeks
Post exposure period : Variable - animals held following exposures until week 96 or 105
Doses : 0, 10, 70, 140, 280, 700, 2200 ppm
Control group : Yes
NOAEL : = 10 ppm
LOAEL : = 70 ppm
Method : other
Year : 1996
GLP : Yes
Test substance : other TS: Isoprene

Method : Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test.

Remark : Control group and treatment: 50 male and 50 female mice exposed to air only

Result : Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks - surviving mice in these groups were necropsied during week 96. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks.

Test condition : Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice.

Test substance : Isoprene (CAS# 78-79-5) purity > 99.0%.

Conclusion : In this study no significant signs of general toxicity were observed. However, a concentration related effect on survival was observed.

Reliability : (1) valid without restriction
29.07.2005 (61)

Type :
Species : Mouse
Sex :
Strain : Wistar
Route of admin. : Inhalation
Exposure period : 4 months
Frequency of treatm. : 5 days/week, 4 hours/day
Post exposure period : 1 month
Doses : 0.011 and 0.12 mg/l
Control group : no data specified

Result : No data on number of animals.

In the low dose group no changes during treatment observed. After the 1 month post observation period the mitotic index in the thymus was significantly raised.

In the high dose group during several phases of the treatment the cell counts of the thymus were significantly increased or decreased. Simultaneously, the thymus weight was significantly increased or decreased. The mitotic index was decreased, but came to a normalized level during the posttreatment observation of 1 month.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (44)

Type :
Species : Rat
Sex : no data
Strain : Fischer 344
Route of admin. : Inhalation
Exposure period : 6 months
Frequency of treatm. : 5 days/week, 4 hours/day
Post exposure period : yes, animals held for 6 months prior to sacrifice
Doses : 0, 70, 220, 700, 2200, 7000 ppm
Control group : Yes
Method :
Year :
GLP : Yes
Test substance :

Result : 12% mortality in 7000 ppm group

Rats showed no effects other than marginal increased incidence of interstitial cell benign adenoma, a common spontaneous lesion in this strain.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
11.01.2005 (55)

Type :
Species : Mouse
Sex : no data
Strain : B6C3F1
Route of admin. : Inhalation
Exposure period : 6 months

Frequency of treatm.	:	5 days/week, 4 hours/day
Post exposure period	:	yes, animals held for 6 months prior to sacrifice
Doses	:	0, 70, 220, 700, 2200, 7000 ppm
Control group	:	Yes
Method	:	
Year	:	
GLP	:	Yes
Test substance	:	
Result	:	12% mortality in 7000 ppm group
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

11.01.2005 (55)

Type	:	
Species	:	Rat
Sex	:	
Strain	:	
Route of admin.	:	Inhalation
Exposure period	:	5 months
Frequency of treatm.	:	4 hours/day
Post exposure period	:	No
Doses	:	2.2 - 4.9 mg/l air in the inhalation chamber
Control group	:	no data specified
Method	:	
Year	:	
GLP	:	No
Test substance	:	
Remark	:	No data on control groups.
Result	:	The studies mainly showed a depression of the lymphatic system and after 3 months a reduced O2-consumption. Histopathological findings: irritation of the bronchia, lung damages, irritation of the thyroid gland
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (24)

Type	:	
Species	:	Mouse
Sex	:	
Strain	:	
Route of admin.	:	Inhalation
Exposure period	:	4 months
Frequency of treatm.	:	4 hours/day
Post exposure period	:	No
Doses	:	2.2 - 4.9 mg/l air in the inhalation chamber
Control group	:	no data specified
Method	:	
Year	:	
GLP	:	No
Test substance	:	
Remark	:	No data on control groups.
Result	:	Histopathological findings: degenerative changes in the liver
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (24)

Type	:	
Species	:	Rabbit
Sex	:	
Strain	:	
Route of admin.	:	Inhalation
Exposure period	:	4 months
Frequency of treatm.	:	4 hours/day
Post exposure period	:	No
Doses	:	2.2 - 4.9 mg/l air in the inhalation chamber
Control group	:	no data specified
Method	:	
Year	:	
GLP	:	No
Test substance	:	
Remark	:	No data on control groups.
Result	:	At the end of the treatment the rabbits showed increased leucocyte counts, decreased erythrocyte counts and increased organ weights. Histopathological findings: damage of the myocard
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (24)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Bacterial
Test concentration	:	0, 100, 333, 1000, 3333, 10000 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	Negative
Method	:	EPA OTS 798.5265
Year	:	1986
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	A positive response was defined as a reproducible, dose-related increase in revertant colonies in any one strain/activation combination. There was no minimum percentage or fold increase required for the chemical to be judged positive or weakly positive.
Remark	:	Species/Strain: Salmonella / TA98, TA100, TA1535, TA1537 Species and cell type: Rat and hamster liver S9 fraction Quantity: 0.5 ml/plate Induced or not induced: Arochlor 1254-induced (500 mg/kg for 5 days)
Result	:	Isoprene was not mutagenic in any of the five strains of Salmonella tested in the presence or absence of Aroclor-induced rat or hamster liver S9. There was no dose-related reproducible increase in the number of revertants in any of the 4 strains tested.
Test condition	:	The preincubation modification of the Salmonella/mammalian microsome assay was used to test isoprene in five different Salmonella strains in the presence and absence of rat and hamster liver S-9. Five dose levels were tested , with three plates per dose level. The high dose was limited by toxicity to 10,000 ug/plate. Concurrent positive controls were also tested with and without metabolic activation. The positive control substances used were: sodium azide for TA1535 and TA100; 4-nitro-o-phenylenediamine for

TA98; and 9-aminoacridine for TA1537; 2-aminoanthracene was used with all strains with hamster and rat liver metabolic activation systems. The assay was repeated less than one week after completion of the initial test.

Test substance : Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion : Isoprene was not mutagenic in the Ames Salmonella mutagenicity test.
Reliability : (1) valid without restriction
Evaluated as part of a NTP-sponsored interlaboratory study of 270 chemicals.

29.07.2005 (51)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, 100, 1530, 1535, 1538
Test concentration : 25 %v/v in the atmosphere, for TA 1538 up to 75 %v/v
Cycotoxic concentr. :
Metabolic activation : with and without
Result : Negative
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (13)

Type : Ames test
System of testing : Salmonella typhimurium TA 102, 104
Test concentration :
Cycotoxic concentr. :
Metabolic activation : Without
Result : Negative
Method :
Year :
GLP : no data
Test substance :

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (39) (52)

Type : other: Ames test with metabolites of isoprene
System of testing : Salmonella typhimurium TA 98, 100
Test concentration : 136 - 2043 µg/plate
Cycotoxic concentr. :
Metabolic activation : Without
Result : Ambiguous
Method :
Year :
GLP : no data
Test substance :

Remark : Tested metabolites:
(I) 3,4-Epoxy-3-methyl-1-butene
(II) 3,4-Epoxy-2-methyl-1-butene
(III) 2-Methyl-1,2,3,4-diepoxybutane

I and II: no mutagenic properties, but at 2043 µg/plate cytotoxic effects
III: dose-dependent increased number of revertants

III has strong alkylating properties

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (23)

Type : other: Ames bacterial reverse mutation test
System of testing : Enclosed static vapor phase exposure chambers
Test concentration : 0, 0.25 to 25% isoprene (2,500 to 250,000 ppm) in vapor phase
Cycotoxic concentr. :
Metabolic activation : with and without
Result : Negative
Method : other: Similar to methods described in OECD 471, and by Green (1984)
Year : 2003
GLP :
Test substance : other TS: Isoprene (CAS No. 78-79-5) Purity 99%

Method : A positive response is defined as reproducible, dose-related increase in revertant colonies in any one strain. An increase of 2x over background is required for a positive response in Salmonella strains TA98 & TA100 and for E. coli bacteria, and 3x background for Salmonella TA1535 & TA1537.
Remark : Species/Strain: Salmonella typhimurium TA98, TA100, TA1535, TA1537, Escherichia coli WP2 uvrA (pKM101)

Species and Cell Type: B6C3F1 mouse liver S9 and microsomal fractions. Source - Molecular Toxicology, Boone, North Carolina

Quantity: 0.24 & 0.47 mg protein (microsomes), and 1.67 mg protein (S-9) added per plate
Result : Negative. Based upon lack of dose-responsiveness and relative revertant scores <2.
The highest increases of isoprene-induced revertant rates relative to background were 1.3-1.7 in TA 1535, and 0.6-1.6 in E. coli, both in presence of S-9. The positive control, VC, induced relative rates of 22-34, and 4.4-6.3, respectively, in these assays. Due to lack of dose-responsiveness, and the fact all revertant rates for isoprene were < 1.7, the criteria for a positive response were not met.
Test condition : The Salmonella/mammalian liver enzyme assay was used to test isoprene in four Salmonella & one E. coli strain in the presence of mouse liver S-9 and microsomes. Isoprene vapor exposures above 25% posed significant cytotoxicity based upon a pilot study with exposures up to 100% isoprene. Exposure levels were tested in three plates per dose level. The concurrent positive control, vinyl chloride monomer (VC), was tested at 2% to confirm both bacterial mutagenic and metabolic activating capacities for liver enzymes.
Test substance : other TS: Isoprene (CAS No. 78-79-5) Purity 99%
Conclusion : Isoprene was not mutagenic in the Ames bacterial mutagenicity test.
Reliability : (1) valid without restriction
Evaluated at Huntingdon Life Sciences (UK) as part of IISRP-sponsored study of olefinic chemicals.

10.12.2003 (26) (32)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary (CHO) cells
Test concentration : 50, 160, 500, 1600 ug/ml (without S9), or 160, 500, 1600, 5000 ug/ml (with S9)
Cycotoxic concentr. :
Metabolic activation :
Result : Negative
Method : OECD Guide-line 479
Year : 1987

GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A frequency 20% above the solvent control group was considered positive. Positive trend tests ($p<0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Remark	:	Induced or not induced: Aroclor 1254-induced Sprague-Dawley rat liver S9. Control groups and treatment: Solvent controls -- dimethylsulfoxide; positive controls -- Mitomycin-C (without S9), cyclophosphamide (with S9).
Result	:	No increases in SCEs were noted in cultured CHO cells treated with isoprene, with or without S9.
Test condition	:	Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Fifty 2nd-division metaphase cells were scored for frequency of SCEs/cell from each dose level.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	Isoprene did not induce sister chromatid exchanges in vitro in cultures of Chinese hamster ovary cells.
Reliability	:	(1) valid without restriction Evaluated as part of a NTP-sponsored study of 108 chemicals.

29.07.2005 (21)

Type	:	Chromosomal aberration test
System of testing	:	Chinese hamster ovary (CHO) cells
Test concentration	:	1600, 3000, 5000 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	:	Negative
Method	:	OECD Guide-line 473
Year	:	1987
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A statistically significant ($p<0.05$) difference for one point and a significant trend ($p<0.015$) was considered positive. Positive trend tests ($p<0.05$) in the absence of a significant difference at any one dose were considered equivocal.
Remark	:	Induced or not induced: Aroclor 1254-induced Sprague-Dawley rat liver S9. Control groups and treatment: Solvent control -- dimethylsulfoxide; positive controls -- Mitomycin-C (without S9), cyclophosphamide (with S9).
Result	:	No increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.
Test condition	:	Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and three doses of isoprene. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.

Conclusion	:	Isoprene did not induce chromosomal aberrations in vitro in cultures of Chinese hamster ovary cells.
Reliability	:	(1) valid without restriction Evaluated as part of a NTP-sponsored study of 108 chemicals.

29.07.2005 (21)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Sister chromatid exchange assay
Species	:	Mouse
Sex	:	Male
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day for 12 days
Doses	:	0, 438, 1750, 7000 ppm
Result	:	Positive
Method	:	other
Year	:	1988
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	The frequencies of sister chromatid exchanges (SCEs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test.
Result	:	NOAEL (NOEL): < 438 ppm LOAEL (LOEL): 438 ppm Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells at all three dose levels (4.40 at 0 ppm, 14.84 at 438 ppm, 11.61 at 1750 ppm, and 13.98 at 7000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other.. There were no significant clinical signs or mortality throughout the study.
Test condition	:	Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0, 438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of SCE, 5 mice per exposure group were killed 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 98%.
Conclusion	:	Isoprene was found to be genotoxic and cytotoxic to mouse bone marrow in vivo - inducing SCE, inhibiting cellular proliferation, and suppressing the rate of erythropoiesis. The lack of significant difference in SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species.
Reliability	:	(1) valid without restriction NTP-sponsored study.

10.12.2003 (73)

Type	:	other: Mammalian Bone Marrow Chromosomal Aberration Test
Species	:	Mouse

Sex	:	Male
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day for 12 days
Doses	:	0, 438, 1750, 7000 ppm
Result	:	Negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year	:	1988
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	The frequencies of chromosomal aberrations (Abs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test.
Result	:	NOAEL (NOEL): 7000 ppm LOAEL (LOEL): > 7000 ppm Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations (Abs) in bone marrow cells. The incidence of bone marrow cells with chromosomal aberrations (Abs) was slightly elevated in the exposed groups compared to the control (0.02 at 0 ppm vs. 0.04, 0.05, and 0.04 at 438, 1750, and 7000 ppm), but these increases were not statistically significant. Mitotic index data indicated no significant change in the percentage of bone marrow cells engaged in division, although the 7000 ppm group was slightly increased compared to the contols (1.15% vs 1.30%). Analysis of average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group (13.72 hours at 7000 ppm vs.11.68 hours at 0 ppm).
Test condition	:	Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0, 438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of Abs, 10 mice per exposure group were killed 17-20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for Abs from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1000 cells/sample was used to calculate the mitotic index.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 98%.
Conclusion	:	The incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days were slightly elevated at all dose groups compared to the controls, but were not statistically increased.
Reliability	:	(1) valid without restriction NTP-sponsored study.

04.04.2003 (73)

Type	:	other: Mammalian Erythrocyte Micronucleus Test
Species	:	Mouse
Sex	:	Male
Strain	:	B6C3F1

Route of admin. : Inhalation
Exposure period : 6 hours/day for 12 days
Doses : 0, 438, 1750, 7000 ppm
Result : Positive
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 1988
GLP : Yes
Test substance : other TS: Isoprene

Method : The number of micronucleated erythrocytes (MN) were summed across animals within each group and analyzed for increasing trend by a one-tailed trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using a one-tailed Pearson Chi square test to determine the minimal effective dose.

Result : NOAEL (NOEL): < 438 ppm
LOAEL (LOEL): 438 ppm

Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure levels tested. The frequencies of MN-PCEs were 2.00, 12.00, 15.60, and 16.93 at 0, 438, 1750, and 7000 ppm. The responses at the 1750 and 7000 ppm levels both were greater than the 438 ppm level, but not statistically different from each other. There also was a dose-related decrease in the percentage of PCEs, a measure of the rate erythropoiesis (3.91, 3.00, 2.87, and 1.64 at 0, 438, 1750, and 7000 ppm). There were no significant clinical signs or mortality throughout the study.

Test condition : Approximately 24 hours following the last exposure peripheral blood samples were obtained from each animal by tail snip, immediately air-dried and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity.

Test substance : Isoprene (CAS# 78-79-5) purity > 98%.

Conclusion : Isoprene was found to be genotoxic to mouse bone marrow in vivo by inducing increased MN in the peripheral blood of male mice. Suppression of erythropoiesis was suggested by decreased percentage of PCEs.

Reliability : (1) valid without restriction
NTP-sponsored study.

07.01.2005 (73)

Type : other: Rat Lung Fibroblast Micronucleus Test
Species : Rat
Sex : male/female
Strain : Fischer 344
Route of admin. : Inhalation
Exposure period : 6 hours/day, 5 days/week for 4 weeks
Doses : 0, 220, 700, or 7000 ppm
Result : Negative
Method : other
Year : 1997
GLP : Yes
Test substance : other TS: Isoprene

Method : Means, standard deviations, and standard error of the mean for the number of mononucleated cells/1000 binucleated cells and micronuclei/1000 binucleated cells were calculated. A two-way analysis of variance was used to analyze the measurements. Intergroup differences were delineated by

Tukey's studentized range test.

Result : There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.

Test condition : This study was performed in conjunction with a two-year carcinogenicity study. Groups of 10 male and 10 female rats (approximately 6-7 weeks old) per group were exposed for 4 weeks (17-19 total exposures) to 0, 220, 700, or 7000 ppm of isoprene by inhalation. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained (acridine orange), and 1000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.

Test substance : Isoprene (CAS# 78-79-5) purity > 99.7%.

Conclusion : No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.

Reliability : (1) valid without restriction
Non-standard method, but comparable to guideline study. Conducted as part of NTP two-year carcinogenicity study.

11.01.2005 (54)

Type : Micronucleus assay
Species : Mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : Inhalation
Exposure period : 20, 40 or 80 weeks
Doses : 0, 10, 70, 140, 280, 700, 2200 ppm/ 4 or 8 hours/day, 5
Result :
Method :
Year :
GLP : Yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Induction of micronuclei after chronic inhalation exposure.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.04.2003 (62)

Remark : Refers to the study that was described above.
Result : When the results of this study were compared to results for butadiene using the same end points, route of exposure and strain, species and sex of animals, isoprene was considered to be of lower genetic toxicity despite being tested at substantially higher concentrations.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (65)

5.7 CARCINOGENICITY

Species : Rat
Sex : male/female
Strain : Fischer 344
Route of admin. : Inhalation

Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week for 104 weeks
Post exposure period	:	None
Doses	:	0, 220, 700, or 7000 ppm
Result	:	Positive
Control group	:	Yes
Method	:	other
Year	:	1997
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed.
Remark	:	Control group treatment: 50 male and 50 female rats exposed to air only.
Result	:	The incidences of mammary gland fibroadenoma in 7,000 ppm males (7/50) and in all groups of exposed females (12/50, 19/50, 17/50) were significantly greater than those in the chamber control groups (1/50 males, 7/50 females). The incidences of renal tubule adenoma in 7,000 ppm males (6/50) and of renal tubule hyperplasia in 700 ppm and 7,000 ppm males (6/50, 8/50) were significantly greater than those in the chamber controls (0/50). The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls. An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats (33/50, 37/50, 44/50, 48/50). The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in the 700 ppm and 7,000 ppm (37/50, 48/50) males were significantly greater than in the chamber controls (20/50). Single incidences of several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brains of female rats in all three exposure groups. The incidences of splenic fibrosis in the 700 and 7,000 ppm males (24/50, 22/50) were significantly greater than that in the chamber control group (11/50).
Test condition	:	Groups of 50 rats/sex /group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104 weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99.7%.
Conclusion	:	Isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the carcinogenicity of isoprene in rats is, at most, limited. However, the NTP concluded that: there was clear evidence of carcinogenic activity in male rats based on increased incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma; some evidence of carcinogenic activity in female rats based on increased incidences and multiplicity of mammary galnd fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene.
Reliability	:	(1) valid without restriction

11.01.2005 (54)

Species	:	Mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	4 or 8 hours/day
Frequency of treatm.	:	Variable - 5 days/week for 20, 40 or 80 weeks
Post exposure period	:	Variable - animals held following exposures until week 96 or 105
Doses	:	0, 10, 70, 140, 280, 700, or 2200 ppm
Result	:	Positive
Control group	:	Yes
Method	:	other
Year	:	1996
GLP	:	Yes
Test substance	:	other TS: isoprene
Method	:	Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test. Incidences of tumor types were analyzed using Fischer's exact test applied to each combination of exposure group and tumor type.
Remark	:	Control group and treatment: 50 male and 50 female mice exposed to air only.
Result	:	The carcinogenic potential of isoprene was evaluated as a function of concentration, length of daily exposure, and weeks of exposure as independent variables. Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks - surviving mice in these groups were necropsied during week 96. Isoprene exposure caused an increase in neoplasms of the lung, liver, Harderian gland, forestomach, lymphoreticular system of male mice and in the Harderian gland and pituitary gland of female mice at concentrations of 70 ppm and higher. The product of concentration and length/duration of exposure was not a sufficient basis for prediction of tumor risk. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks (the 280 and 700 ppm groups were not sampled by protocol design).
Test condition	:	Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice.
Conclusion	:	The results of this study indicated that concentration, , length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. There appeared to be threshold for oncogenic effects in mice, which varied by organ and tumor type. For male mice, the LOEL was 700 ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 pmm for Harderian gland tumors. For female mice, the LOEL was 70 ppm for total non-liver, non-lung adenomas and possibly for hemagiosarcomas.
Reliability	:	(1) valid without restriction

29.07.2005 (61)

Species	:	Mouse
Sex	:	Female
Strain	:	ICR
Route of admin.	:	Dermal
Exposure period	:	18 weeks after a 3 week initiation with DMBA
Frequency of treatm.	:	5 times/week
Post exposure period	:	no information
Doses	:	1.5 % isoprene and 0.04 % crotone oil (mixture)
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: initiation/promotion study
Year	:	1971
GLP	:	No
Test substance	:	
Remark	:	Study inadequate to assess the carcinogenic effects of isoprene.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (64)

Species	:	Mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	40 or 80 weeks
Frequency of treatm.	:	4 or 8 hours/day, 5 days/week
Post exposure period	:	24 weeks after 80 weeks treatment->necropsy
Doses	:	0, 10, 70, 140, 280, 700, 2200 ppm
Result	:	
Control group	:	Yes
Method	:	other
Year	:	
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Study designed to determine the oncogenic potential as a function of exposure level, length of daily exposure and exposure duration. To achieve this 15 groups of 50 animals each were exposed to various concentrations of isoprene (0-2200 ppm), 4 to 8 hours daily for 40 or 80 weeks. There was an exposure related increase in tumours at multiple sites. The tumour pattern was similar to the profile observed in butadiene, 1,3- with the exception of the early onset of T-cell lymphoma in butadiene treated mice. Biostatistical analyses indicated, that the product of concentration and length/duration of exposure was not a sufficient basis for predicting tumour risk at any site. Extrapolation of tumour probability between the high and low concentrations based on cumulative exposure was not appropriate and could not be justified by statistical models.
Source	:	Deutsche Shell Chemie GmbH Eschborn Exxon Chemical Europe Inc. Bruxelles

11.01.2005 (62)

5.8.1 TOXICITY TO FERTILITY

Type	:	other: 13-week inhalation study
Species	:	Rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	13 weeks
No. of generation studies	:	
Doses	:	0, 70, 220, 700, 2200 or 7000 ppm
Control group	:	Yes
NOAEL parental	:	= 2200 ppm
Method	:	other
Year	:	1994
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Analysis of incidence of neoplastic and nonneoplastic lesions was performed.
Remark	:	Control group and treatment: 10 male and 10 female rats exposed to air only.
Result	:	There were no exposure-related effects in rats except a slight increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm group.
Test condition	:	Groups of 10 animals/sex/group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility and vaginal cytology were performed on all rats exposed to 0, 70, 700 or 7000 ppm isoprene. Histopathologic evaluations of the reproductive organs were performed on all rats as part of the terminal sacrifice for the core 13-week subchronic inhalation study.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	No significant effects on reproductive endpoints were observed in rats except slight changes in the testis at the highest exposure level (7000 ppm).
Reliability	:	(2) valid with restrictions Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study.

29.07.2005 (49)

Type	:	other: 13-week inhalation study
Species	:	Mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	Inhalation
Exposure period	:	6 hours/day
Frequency of treatm.	:	5 days/week
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	13 weeks
No. of generation studies	:	
Doses	:	0, 70, 220, 700, 2200 or 7000 ppm

Control group	:	Yes
NOAEL parental	:	= 220 ppm
Method	:	other
Year	:	1994
GLP	:	Yes
Test substance	:	other TS: Isoprene
Method	:	Analysis of incidence of neoplastic and nonneoplastic lesions was performed.
Remark	:	Control group and treatment: 10 male and 10 female mice exposed to air only.
Result	:	In this study the testicular weight of male mice was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice. Males in the 700 and 7000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days).
Test condition	:	Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility and vaginal cytology were performed on all mice exposed to 0, 70, 700 or 7000 ppm isoprene. Histopathologic evaluations of the reproductive organs were performed on all and mice as part of the terminal sacrifice for the core 13-week subchronic inhalation study.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99%.
Conclusion	:	Mice exhibited significant effects at 700 ppm or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility.
Reliability	:	(2) valid with restrictions Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study.

29.07.2005 (49)

Type	:	One generation study
Species	:	Rat
Sex	:	Female
Strain	:	Wistar
Route of admin.	:	oral unspecified
Exposure period	:	4 days
Frequency of treatm.	:	not specified
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	22, 380, 1900 mg/kg b. w.
Control group	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Remark	:	Female rats received the oral between day 9 and 12 of gestation.
Result	:	No indication of embryotoxic or teratogenic effects. The fetusses showed slightly retarded ossification of the

sternum and the occiput.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (74)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	gestation days 6-19
Frequency of treatm.	:	6 hours/day
Duration of test	:	Females sacrificed on gestation day 20
Doses	:	0, 280, 1400, or 7000 ppm
Control group	:	other: yes (10 virgin females)
NOAEL maternal tox.	:	= 7000 ppm
NOAEL teratogen.	:	= 7000 ppm
Method	:	EPA OTS 798.4350
Year	:	1989
GLP	:	Yes
Test substance	:	other TS: Isoprene
Remark	:	Control group and treatment: air-exposed only
Result	:	Maternal effects: Exposure of pregnant rats to these concentrations of isoprene did not result in apparent maternal toxicity. The only effect observed in the rat dams was an increased kidney to body weight ratio at the highest level (7000 ppm). Embryo/fetal effects: In rats, there was no adverse effect on any reproductive index at any level and there was no increase in fetal malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm.
Test condition	:	Approximately 30 positively mated rats were exposed on days 6-19 of gestation. The day of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99.7%.
Conclusion	:	Pregnant Sprague-Dawley rats and their offspring exhibited no significant toxic effects of isoprene at any exposure level in this study.
Reliability	:	(1) valid without restriction NTP-sponsored study.

10.01.2005 (53)

Species	:	Mouse
Sex	:	Female
Strain	:	CD-1
Route of admin.	:	Inhalation
Exposure period	:	Gestation days 6-17
Frequency of treatm.	:	6 hours/day
Duration of test	:	Females sacrificed on gestation day 18
Doses	:	0, 280, 1400, or 7000 ppm
Control group	:	Yes
NOAEL maternal tox.	:	= 1400 ppm
NOAEL teratogen.	:	< 280 - ppm

Method	:	EPA OTS 798.4350
Year	:	1989
GLP	:	Yes
Test substance	:	other TS: Isoprene
Remark	:	Control group and treatment: air-exposed only
Result	:	Maternal effects: Exposure of Swiss (CD-1) mice to isoprene resulted in (from day 12 onward) significant reductions in maternal body weight, body weight gain during treatment, and in uterine weight for the 7000 ppm group. Liver to body weight ratios for pregnant mouse dams were significantly increased in the 1400 and 7000 ppm groups compared to the control group, and kidney to body weight ratios were significantly increased the 7000 ppm group. Embryo/fetal effects: In mice, there was an exposure-related and statistically significant reduction in fetal body weights at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. There was no significant increase in the incidence of fetal malformations or ossifications, although two fetuses with cleft palate were found, one in each of the two highest exposure groups (1400 and 7000 ppm). Cleft palates were not detected in the control group. Increased incidences of variations (supernumerary ribs) were observed in the exposed groups, although this skeletal variation is generally considered a secondary effect of maternal toxicity or stress and it's significance is unclear. The incidence of supernumerary ribs (percent of fetuses examined) was 20.1, 23.8, 33.6, and 40.3% at 0, 280, 1400, and 7000 ppm.
Test condition	:	Approximately 30 positively mated mice were exposed on days 6-17 of gestation. The day of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects.
Test substance	:	Isoprene (CAS# 78-79-5) purity > 99.7%.
Conclusion	:	Swiss (CD-1) mouse dams exhibited significant toxic effects only at the 7000 ppm level; however the offspring exhibited significant signs of toxicity, including reductions in fetal body weight which were statistically significant at the 280 ppm level (lowest isoprene level) for female fetuses and at the 1400 ppm level for male fetuses.
Reliability	:	(1) valid without restriction NTP-sponsored study.

10.01.2005 (53)

Species	:	Rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	day 6-19 of gestation
Frequency of treatm.	:	6 hours/day, 7 days/week
Duration of test	:	12/14 days
Doses	:	0, 280, 1400, 7000 ppm
Control group	:	Yes
Method	:	
Year	:	1989
GLP	:	no data
Test substance	:	
Remark	:	No adverse effect on the dams or on any reproductive index at any dose level.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (45) (46)

Species : Mouse
Sex : male/female
Strain : CD-1
Route of admin. : Inhalation
Exposure period : day 6-17 of gestation
Frequency of treatm. : 6 hours/day, 7 days/week
Duration of test : 12/14 days
Doses : 0, 280, 1400, 7000 ppm
Control group : Yes
Method :
Year : 1989
GLP : no data
Test substance :

Remark : 7000 ppm induced reduction in maternal weight gain; reduced fetal body weight at all dose levels. No fetal malformations except extra ribs at 7000 ppm.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (45) (46)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Remark : Toxic effects observed in humans:

catarrhal inflammation, subtrophic and atrophic processes in thr upper respiratory tract, deterioration of olfaction were noted in isoprene rubber production workers.. Incidence and degree was correlated with duration of occupation.
Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (50) (63)

5.11 ADDITIONAL REMARKS

Type : Metabolism

Remark : Isoprene is metabolized via a similar mechanism as that for butadiene. However, unlike butadiene, the monoepoxide intermediates of isoprene are not mutagenic. Only the minor epoxide (20 % of the total) is further oxidized to the mutagenic diepoxide. Furthermore, liver microsomes from mice and hamsters showed a six-fold higher maximum metabolic

velocity (Vmax) than those from rats and rabbits (Longo et al., 1985). This species-difference was further demonstrated using a two-compartment model of isoprene pharmacokinetics. Both rats and mice exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm. However, the Vmax in mice was determined to be 400 µmol/h/kg or more than three times that in rats (130 µmol/h/kg) implying a species-sensitivity to diepoxide formation in the mouse.Endogenous isoprene production rate was determined to be 1.9 and 0.4 µmol/h/kg in rats and mice, respectively (Peter et al., 1987).

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (41) (58)

Type : Metabolism

Remark : This paper describes comparative studies on the stereochemistry of the metabolism of isoprene that were carried out in vitro using liver microsomes from rats, mice, monkeys, dogs, rabbits and humans. Differences between strains and gender were also investigated. In the production f the isoprene monoepoxides, microsomes from the livers of the male Sprague-Dawley or Wistar rat showed an approximately 2:1 preference for the formation of (S)-2-(1-methylethenyl)oxirane compared with the (R)-enantiomer. No enantioselectivity was observed for mouse or rabbit. In contrast, liver microsomes from dog, monkey or male human preferentially formed (R)-2(1-methylethenyl)oxirane. There was no enantioselectivity observed with microsomes from female human liver. In conclusion, the significant differences between species in the in vitro metabolism of isoprene indicate that stereochemical and mechanistic data should be taken into account when evaluating the results of animal studies designed to assess the carcinogenic risks to humans that may be associated with exposure to isoprene.

29.07.2005 (70)

Type : Metabolism

Remark : This study evaluated the stereoselectivity of the in vitro conversion of isoprene by liver enzymes of rats and mice. Reaction mixtures (0.5 mL) containing rat or mouse liver microsomes (1nmol of cytochrome P450) along with buffer and other co-factors were incubated with isoprene (10 umol) for 130 minutes at 37 degrees C. The percentage of the monooxirane enantiomers formed by the enzymatic isoprene epoxidation were determined by complexation gas chromatography by the head-space technique.

Isoprene was epoxidized by cytochrome P450 of rats and mice to 2-isopropenyloxirane and 2-methyl-2-vinyloxirane with slight but different product enantioselectivity. Only with mouse liver microsomes was a distinct regioselectivity observed. Both monooxiranes were further epoxidized to 2-methyl-2,2'-bioxirane with substrate enantioselectivity, product diastereoselectivity, and with product enantioselectivity. The epoxide hydrolase-catalyzed hydrolysis with rat and mouse liver microsomes occurs with substrate enantioselectivity. The epoxide hydrolase-catalyzed hydrolysis with rat and mouse liver microsomes occurs with substrate enantioselectivity. A better kinetic resolution was found for 2-isopropenyloxirane than for 2-methyl-2-vinyloxirane. While 2(R)-isopropenyloxirane was conjugated preferentially with glutathione, catalyzed by glutathione S-transferase, no enantiomer differentiation takes place in the case of 2-methyl-2-vinyloxirane.

29.07.2005 (77)

Type : Metabolism

Remark : Isoprene is one of the main constituents of endogenous origin in exhaled human breath. In this study, breath samples were collected from fifty volunteers, 30 women and 20 men, aged 15 to 60 years, at various times of the day. Twenty-five of them also collected samples at different moments of the night, either while entirely awake or just after being awakened and allowed to fall asleep again. For practical purposes, samples obtained immediately after spontaneous or induced awakening were considered to be identical to those that might have been collected during sleep.
The concentration of isoprene in the breath taken at different moments of the daytime period , between 8 and 23 hours, from 50 healthy volunteers while fully awake was 14.6 + 6.4 nmol/L. This result is in agreement with other published values and demonstrates that the elimination of isoprene does not appreciably change during the diurnal period in individuals who stay awake. The isoprene concentration in the individuals awake during the night (i.e., 17.7 + 7.0 nmoles/L) was similar to that in individuals awake during the day (14.6 + 6.4 nmoles/L). The isoprene concentration in the 13 subjects who were allowed to sleep was significantly higher than in the 9 subjects who stayed awake. Thus, in the absence of sleep during the night, the concentration of isoprene in the breath did not increase. On awakening in the morning or in the middle of the night, isoprene concentration was observed to fall sharply in less than 20 minutes. The isoprene concentration decreased from 42.4 + 13.5 to 18.2 + 4.7 nmoles/L (n=6) at 0200 hours and from 45.3 + 16.5 to 23.3 + 7.7 nmoles/L (n=14) at 0600 hours.
In conclusion, this study demonstrates that the concentration of isoprene varies with states of sleep and wakefulness, increasing during sleep and decreasing sharply just after awakening.

29.07.2005 (6)

Type : Metabolism

Remark : The present study investigated the metabolism of isoprene by the mouse liver cytochrome P-450 system to quantitatively determine the formation of possibly genotoxic epoxide intermediates. This study demonstrated that mouse liver microsomal mono-oxygenases metabolize isoprene to the corresponding mono-epoxides. The reaction was shown to be dependent on NADPH and oxygen and was inhibited by carbon monoxide, metyrapone and SKF52S-A. Of the two epoxides formed, 3,4-epoxy-3-methyl-1-butene was the major metabolite (approximately 80% formed) whereas 3,4-epoxy-2-methyl-1-butene was the minor metabolite (approximately 20% formed). The minor metabolite, 3,4-epoxy-2-methyl-1-butene was further epoxidated to the mutagenic and presumably carcinogenic isoprene diepoxide.

29.07.2005 (14)

Type : Toxicokinetics

Remark : The purpose of this study was to determine the toxicokinetics of inhaled isoprene in B6C3F1 mice and to compare the data to previously published toxicokinetic data inF344 rats. Male B6C3F1 mice were exposed to nominal concentrations of 20, 200, and 2000 ppm isoprene or [14C] isoprene for up to 6 hours. For all exposures, steady-state levels of isoprene were reached rapidly (i.e., within 15 to 30 minutes) after the onset or exposure.

There were substantial differences in the toxicokinetics of inhaled isoprene in mice compared to rats. In mice, fractional retention of inhaled isoprene, which reflects, in part, metabolism of isoprene, was linearly related to exposure concentrations up to 200 ppm but decreased at 2000 ppm; in rats, fractional retention of inhaled isoprene decreased with increasing exposure concentration over a range of exposures from 8 to 1500 ppm. Rats metabolized a greater fraction of the inhaled isoprene than did mice at all exposure concentrations. The differences in uptake and disposition between the two species should be considered in extrapolation of rodent data to humans.

29.07.2005 (4)

Type : Toxicokinetics

Remark : A physiological toxicokinetic (PT) model was developed for isoprene in mouse, rat and human. Experimentally determined partition coefficients were taken from the literature. Metabolic parameters were obtained from gas-uptake experiments. The measured data could be described by introducing hepatic and extrahepatic metabolism into the model. At exposure concentrations up to 50 ppm, the rate of metabolism at steady-state is 14 times faster in mice and about 8 times faster in rats than in humans. Isoprene accumulated only barely due to its fast metabolism and its low thermodynamic partition coefficient whole body:air. In addition, isoprene is produced endogenously. This production is negligible in rodents compared to that in humans (0.34 mmol /h/kg). About 90% of isoprene produced endogenously in humans is metabolized and 10% is exhaled unchanged. The blood concentration of isoprene in non-exposed humans is predicted to be 9.5 nmol/l. The area under the blood concentration-time curve (AUC) following exposure over 8 h to 10 ppm isoprene is about 4 times higher than the AUC resulting from the unavoidable endogenous isoprene over 24 h. A comparison of such AUCs can be used for establishing workplace exposure limits. For estimation of the absolute risk, knowledge of the body burden of the epoxide intermediates of isoprene is required. However, such data are not yet available.

29.07.2005 (11)

Type : Toxicokinetics

Remark : Male Fischer 344 rats exposed by nose-only inhalation for 6 hours to 8, 260, 1480, and 8200 ppm [4-14C] isoprene retained 19, 9, 6, and 5% of the inhaled radio-ac-ti-vity, respectively. About 75% of the retained isoprene radio-acti-vity was excreted in urine within 66 hours. Liver, blood, and especially fat contained the most isoprene and metabolites. During the in-hala-tion phase, respira--tory tract tissues contained concentrations of volatile metabolites substantial-ly out of proportion to their mass relative to liver and blood; this was inter-preted to indicate metabolism in the respiratory tract. Most of the radio-activity in blood (>85%) was associated with material of low volatility, probably mostly con-jugates or tetrols. Between 0.031% (at 8 ppm) and 0.002% (at 8200 ppm) of the inhaled 4-14C label was tentatively identified as isoprene diepoxide. Thus, the relative amount of the metabolites present in rat blood was highest for low con-cen-trations of inhaled isoprene. Under the assumption that all radioactive material with the volatility of the diepoxide was indeed the diepoxide, blood diepoxide con-cen-tra-tions of 0.37, 7.4, 15, and 17 mmol/L were derived from 6-hour exposures to 8, 260, 1480, and 8200 ppm, respectively.

29.07.2005 (12)

Type : Toxicokinetics

Remark : This study describes the in vitro biosynthesis of isoprene from DL-mevalonate in the cytosolic fraction of rat liver. The data presented support the hypothesis that breath isoprene is the result of cellular mevalonate metabolism and arises from the non-enzymatic decomposition of a C5 unit.

29.07.2005 (15)

Type : Toxicokinetics

Remark : In this study, the concentration of isoprene, the main hydrocarbon of human breath, was measured in the blood of humans and in the blood of five different animal species, ie., rat, rabbit, dog, ewe and cow. In human blood, the concentrations of isoprene were between 15 and 70 nmol/liter with a mean value of 37 + 25 nmol/liter. In animals, traces of isoprene were unambiguously detected by mass spectrometry in the blood of all species tested. However, the levels were always lower than 1 nmol/liter.

29.07.2005 (7)

Type : Toxicokinetics

Remark : This study was conducted to provide a better understanding of the mechanisms of reactions of the epoxides of butadiene and isoprene with biologically relevant nucleophiles. The reactivity of the mono-epoxide of butadiene, i.e., ethenyloxirane and the mono-epoxide of isoprene, i.e., 2-ethenyl-2-methyloxirane were compared with oxygen, nitrogen and sulfur nucleophiles. It was discovered that 2-ethenyl-2-methyloxirane unexpectedly suffers cleavage by nitrogen and sulfur nucleophiles preferentially at the "neo pentyl"position C-3. The resulting adducts were obtained as homogeneous compounds and fully characterized by spectroscopic analysis.

29.07.2005 (2)

Type : Toxicokinetics

Remark : The metabolism of isoprene was investigated with microsomes from cell lines expressing eight different human cytochrome P450 enzymes (i.e., CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, CYP3A4). CYP2E1 showed the highest rates of formation of the isoprene monoepoxides 3,4-epoxy-3-methyl-1-butene (EPOX-1) and 3,4-epoxy-2-methyl-1-butene (EPOX-11), followed by CYP2B6. CYP2E1 was the only enzyme showing detectable formation of the diepoxide of isoprene, 2-methyl-1,2:3,4-diepoxybutane. Both isoprene monoepoxides were oxidized by CYP2E1 to the diepoxide at similar enzymatic rates.

To investigate species differences with regard to the role of epoxide hydrolase in the metabolism of isoprene monoepoxides, the epoxidation of isoprene by human liver microsomes was compared to that of mouse and rat liver microsomes. The amounts of monoepoxides formed as a balance between epoxidation and hydrolysis was measured in incubations with and without the epoxide hydrolase inhibitor cyclohexene oxide. Inhibition of epoxide hydrolase resulted in similar rates of monoepoxide formation in mouse, rat and man. Without inhibitor, however, the total amount of monoepoxides present at the end of the incubation period was twice as high for mouse liver microsomes than for rat and even 15 times as high as for human liver microsomes. Thus, differences in epoxide hydrolase activity between species may be of crucial importance for the toxicity of isoprene in the various species.

29.07.2005 (3)

Type : other: Metabolism and Mutagenicity

Remark : This study evaluated the in vitro biotransformation of isoprene by hepatic subcellular fractions from four rodent species, i.e., mouse, rat, rabbit and hamster. Isoprene metabolism showed the same pattern in all species tested. In all cases, isoprene was metabolized to two mono-epoxides (i.e., 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene) and one di-epoxide (i.e., 2-methyl-1,2,3,4-diepoxybutane). Among the epoxide metabolites of isoprene only the di-epoxide proved to be mutagenic in the Ames Salmonella mutagenicity assay in strain TA100. Of note, the isoprene di-epoxide was found to have a half-life and mutagenic and alkylating activities similar to those of the structurally related butadiene di-epoxide. The butadiene di-epoxide has been shown to be mutagenic, clastogenic and carcinogenic.

29.07.2005 (22)

Type : other: Pharmacokinetics

Remark : This study investigated the pharmacokinetics of isoprene in male B6C3F1 mice and male Wistar rats. In a series of experiments conducted with either 2 male Wistar rats or 5 male B6C3F1 mice, animals were exposed to different initial concentrations of gaseous isoprene, up to 4000 ppm in a closed exposure system. Time-dependent concentration decline in the atmosphere of the system was determined by gas chromatography. Similarly, exhalation and accumulation of endogenously produced isoprene was determined in untreated mice and rats. In both species, metabolism of isoprene shows saturation kinetics. Below atmospheric concentrations of 300 ppm in rats and in mice, the rate of metabolism is directly proportional to the concentration. The low accumulation of isoprene in the body at low atmospheric concentrations suggests transport limitation of the metabolism. Only small amounts of isoprene taken up are exhaled as unchanged substance (i.e., 15% in rats and 25% in mice). The half life of isoprene is 6.8 minutes in rats and 4.4 minutes in mice. At concentrations above above 300 ppm the rate of metabolism does not increase further in proportion to the atmospheric concentration. It finally approaches maximal values of 130 mmol / (h x kg) body weight atmospheric concentrations above 1500 ppm in rats, and 400 mmol / (h x g) body weight at concentrations above 2000 ppm in mice. This indicates limited production of the two possible mono-epoxides of isoprene at high concentrations.

Isoprene is endogenously produced and is systemically available. Its production rate is 1.9 mmol / (h x kg) in rats, and 0.4 mmol / (h x kg) in mice, respectively. Part of the endogenous isoprene is exhaled by the animals but it is metabolized to a greater extent: the rate of metabolism of endogenously produced and systemically available isoprene is 1.6 mmol / (h x kg) (rats) and 0.3 mmol / (h x kg) (mice).

29.07.2005 (58)

Remark : The purpose of this study was to obtain comparative pharmacokinetic data on the metabolism of inhaled isoprene in rats and mice. Inhalation studies were conducted in male Wistar rats and male B6C3F1 mice to investigate possible species differences in metabolism of this compound. In these studies two rats or five mice were placed in a closed 6.4 L dessicator jar chamber, equipped with 135 g soda lime for CO2 absorption and an oxygen supply. The animals were exposed to initial concentrations between about 5 ppm and 5000 ppm isoprene. Concentration changes of the compound in the gas phase of the system were measured by gas chromatography. Similarly, exhalation and accumulation of isoprene

endogenously produced by untreated animals while in the closed exposure system was determined. Kinetic parameters were determined from the concentration time-courses thus obtained, based on a two compartment pharmacokinetic model developed by Filser and Bolt.

For rats and mice, linear pharmacokinetics apply at exposure concentrations below 300 ppm isoprene. Saturation of isoprene metabolism is practically complete at atmospheric concentrations of about 1000 ppm in rats and about 2000 ppm in mice. In the lower concentration range where first-order metabolism applies, metabolic clearance of inhaled isoprene per kilogram body weight was 6200 mL/hr for rats and 12,000 mL/hr for mice. The estimated maximal metabolic elimination rates were 130 mmol /hr/kg for rats and 400 mmol /hr/kg for mice. This shows that the rate of isoprene metabolism in mice is about two or three times that in rats.

When the untreated animals are kept in a closed all-glass exposure system, the exhalation of isoprene into the system can be measured. This shows that the isoprene endogenously produced by the animals is systemically available within the animal organism. From such experiments the endogenous production rate of isoprene was calculated to be 1.9 mmol /hr/kg for rats and 0.4 mmol /hr/kg for mice. These data indicate that the endogenous production or isoprene should be accounted for when discussing a possible carcinogenic or mutagenic risk of this compound.

29.07.2005 (59)

Remark : The haemoglobin adduct formation measured after i. p. injection into male Sprague-Dawley rats and male B6C3F1 mice was linearly related to administered dose up to 500 µmol/kg and showed the same slope with both species. Dose correction for isoprene exhalation resulted in haemoglobin adduct formation of 0.16 and0.08 pmol Hb adduct/mg globin per µmol retained isoprene/kg body weight for mice and rats, respectively.

Source : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (72)

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