Screening Assessment for the Challenge

1,3-Butadiene, 2-methyl-
(Isoprene)

Chemical Abstracts Service Registry Number
78-79-5

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment of 1,3-butadiene, 2-methyl- (isoprene), Chemical Abstracts Service Registry Number (CAS RN) 78-79-5. This substance was identified in the categorization of the Domestic Substances List as a high priority for action under the Ministerial Challenge. Isoprene was identified as a high priority as it was considered to pose greatest potential for exposure to individuals in Canada (GPE) and had been classified by other agencies on the basis of carcinogenicity and mutagenicity. The substance did not meet the criteria for persistence, bioaccumulation or inherent toxicity to aquatic organisms. Therefore, the focus of this assessment on isoprene relates to human health aspects.

Under information reported pursuant to Section 71 of CEPA 1999, the total quantity of isoprene manufactured in Canada in 2006 exceeded 10 000 000 kg and the total quantity imported ranged from 1 000 000 to 10 000 000 kg. This substance is used mainly as a monomer in the production of polyisoprene, butyl rubber and styrene-isoprene-styrene (SIS) rubber. Polyisoprene is subsequently used in the production of vehicle tires and a wide variety of products including paint resins, footwear, adhesives and molded goods. . Butyl rubber is typically used in the manufacture of inner tubes, while SIS rubber is used in pressure sensitive adhesives. Isoprene is also used in the formulation of viscosity improvers for motor oil and in the production of agrochemicals, pharmaceuticals and other substances.

Isoprene is emitted into the environment from both natural and anthropogenic sources, and the principal route of exposure for the general population will likely be through inhalation of ambient and indoor air. Off-gassing of isoprene from consumer products manufactured from polyisoprene may also contribute to the levels of the substance in indoor air.

Based principally on the weight of evidence-based assessments of several international and national agencies, a critical effect for the characterization of risk to human health is carcinogenicity, based on observation of tumours at multiple organ sites in rats and mice. Isoprene was also genotoxic in several *in vivo* assays. Therefore, although the mode of action has not been fully elucidated, it cannot be precluded that tumours observed in experimental animals resulted from direct interaction with genetic material.

On the basis of carcinogenicity, for which there may be a probability of harm at any level of exposure, as well as the potential inadequacy of the margin between concentrations of isoprene in indoor air and levels associated with non-cancer effects in the thymus in a subchronic study, it is concluded that isoprene be considered as a substance which may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

On the basis of low ecological hazard and reported releases of isoprene, it is concluded that this substance is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity, or that constitute or may constitute a danger to the environment on which life depends. As set out in the *Persistence and Bioaccumulation Regulations*, isoprene does not meet the criteria for persistence in air, water, soil or sediment, nor does it meet the criteria for bioaccumulation potential.

In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Based on information available, isoprene meets one or more of the criteria set out in section 64 of the *Canadian Environmental Protection Act, 1999*.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or human health. Based on the results of a screening assessment, the Ministers can propose to take no further action with respect to the substance, to add the substance to the Priority Substances List (PSL) for further assessment, or to recommend that the substance be added to the List of Toxic Substances in Schedule 1 of the Act and, where applicable, the implementation of virtual elimination.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherently toxic to aquatic organisms (iT), and were believed to be in commerce; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE), and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006), that challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance 1,3-butadiene, 2-methyl- (isoprene) was identified as a high priority for assessment of human health risk because it was considered to present GPE and had been classified by other agencies on the basis of carcinogenicity and genotoxicity. The Challenge for isoprene was published in the *Canada Gazette* on May 12, 2007 (Canada 2007). A Substance Profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information were received.

Although isoprene was determined to be a high priority for assessment with respect to human health, it did not meet the criteria for potential for bioaccumulation or inherent toxicity for aquatic organisms. Therefore, this assessment focuses principally on information relevant to the evaluation of risks to human health.

Under CEPA 1999, screening assessments focus on information critical to determining whether a substance meets the criteria for defining a chemical as toxic as set out in section 64 of the Act, where

> "64. […] a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that
>
> (*a*) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
>
> (*b*) constitute or may constitute a danger to the environment on which life depends; or
>
> (*c*) constitute or may constitute a danger in Canada to human life or health."

Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.

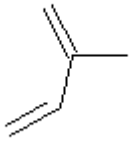
This screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to February 2008 and June 2008 for information on health effects and human exposure, respectively. Key studies were critically evaluated; modelling results may have been used to reach conclusions. Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally on the weight of evidence based assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conculsion is based.

This screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. This assessment has undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including John Christopher (California Department of Toxic Substances Control), Michael Jayjock (The LifeLine Group) and Wendy Heiger-Bernays (The Science Collaborative and Boston University). While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. The critical information and considerations upon which the assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as isoprene.

Table 1. Substance identity

Chemical Abstracts Service Registry Number (CAS RN)	78-79-5
Domestic Substances List (DSL) name	1,3-butadiene, 2-methyl-
National Chemical Inventory (NCI) names[1]	1,3-butadiene, 2-methyl- (TSCA, ENCS, AICS, SWISS, PICCS, ASIA-PAC, NZIoC) isoprene (EINECS, PICCS) 2-Methyl-1,3-butadiene (ECL) BUTA-1,3-DIENE, 2-METHYL- (PICCS)
Other names	β-methylbivinyl; 2-methylbutadiene; 3-methyl-1,3-butadiene; isopentadiene
Chemical group (DSL stream)	Organics
Chemical formula	C_5H_8
Chemical structure	
Simplified Molecular Input Line Entry (SMILES)	**C(C=C)(C)=C**
Molecular mass	68.12 g/mol

[1] National Chemical Inventories (NCI). 2007: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing

Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); NZIoC (New Zealand Inventory of Chemicals); PICCS (Philippine Inventory of Chemicals and Chemical Substances); SWISS (Inventory of Newly Notified Substances and Giflist 1- List of Toxic Substances); and TSCA (Toxic Substances Control Act Chemical Substance Inventory).

Physical and Chemical Properties

A summary of key physical chemical properties for isoprene is presented in Table 2. At room temperature, isoprene is a clear, colourless liquid. It is flammable and highly reactive and is capable of polymerizing explosively when heated.

Table 2. Physical and chemical properties for isoprene

Property	Value	Rating	Reference
Melting point (°C)	-145.9		HSDB 2008
Boiling point (°C)	34.1		HSDB 2008
Density (g/ml at 20°C)	0.68		Merck 1996
Vapour pressure (Pa)	73 327[1]	Very high	HSDB 2008
Henry's Law constant (atm-m^3/mol)	0.077;	Very high	HSDB 2008
Water solubility (mg/L)	642	Moderate	HSDB 2008
Log K_{ow} (Octanol-water partition coefficient; dimensionless)	2.42	Low	HSDB 2008
Log K_{oc} (Organic carbon-water partition coefficient; dimensionless)	2.69	Moderate	HSDB 2008

[1] Converted from 550 mm/Hg

Sources

Isoprene is a biogenic volatile organic compound that is naturally emitted to the atmosphere from various plant and tree species (Zimmer et al. 2000). Indoor house plants may also be a source of exposure, although no relevant data have been identified. The substance is also formed endogenously in humans and is generally the major hydrocarbon present in exhaled breath (Gelmont et al. 1981). Anthropogenic releases occur during the manufacture of synthetic rubber and other elastomers (Lewis 1997). Isoprene may also be released to the environment through rubber abrasion (Graedel 1986).

Further releases occur during wood pulping operations and from oil fires, wood-burning stoves, fireplaces and other biomass combustion processes. Isoprene is also found in

gasoline, cigarette smoke and exhaust gases from turbines and automobiles (HSDB 2008).

Under information reported pursuant to the CEPA 1999 section 71 notice with respect to isoprene, Canadian companies reported manufacturing the substance in a quantity greater than 10 000 000 kg and importing a quantity in the range of 1 000 000 kg to 10 000 000 kg for the 2006 calendar year. However, greater than 10 000 000 kg was exported during the same calendar year. (Environment Canada 2007a).

Uses

According to submissions made under section 71 of CEPA 1999 Challenge questionnaire submissions, other voluntarily submitted data and available scientific and technical literature, isoprene is used mainly as a monomer in the production of polyisoprene (*cis*-1,4-polyisoprene), butyl rubber (isobutene-isoprene copolymer), thermoplastic and elastomeric co-block polymers (e.g., styrene-isoprene-styrene rubber). Polyisoprene is used mostly in the production of vehicle tires and in the manufacture of a wide variety of products including medical equipment, toys, shoe soles, elastic films and threads for textiles and golf balls, adhesives and paints and coatings, while butyl rubber is typically used in the manufacture of inner tubes and styrene-isoprene-styrene rubber is used in pressure sensitive adhesives (Environment Canada 2007a; IARC 1999; OECD 2005). Isoprene may also be used in the formulation of viscosity improvers and in the production of agrochemicals, pharmaceuticals and other chemicals (Shell 2008).

In Canada, a large number of cosmetic products are available that are formulated with polyisoprene or various isoprene copolymers (e.g., styrene/isoprene, isoprene/pentadiene), but no information on the amount of unreacted isoprene in these products is available (as per email from Cosmetic Products Branch, dated June 9, 2008). Butyl rubber is also used in can sealants for food containers and crown corks used on bottles, coatings for plastic films and seam end tops used in packaging in accordance with good manufacturing practice (GMP) where any contact with the food is expected to be incidental (as per email from Health Products and Food Branch, dated Feb 15 2008).

Releases to the Environment

Isoprene is a naturally occurring substance that is continually emitted to the atmosphere by agricultural crops, trees and other vegetation. It is also the basic structural unit in many natural products, including terpenes and vitamins A and K (IARC 1994). It has been estimated that global emissions of the substance range from 1.75 to 5.03 x 10^{11} kg C per year and represent approximately 44–51% of the total global natural volatile organic compound emissions (Guenther et al. 1995).

Anthropogenic releases of isoprene occurring during ethylene production by the cracking of naphtha and from other industrial operations involving the use of isoprene (e.g.,

manufacture of polyisoprene) are much smaller in comparison to natural releases. The National Pollutant Release Inventory (NPRI) reports the releases of isoprene to air from industrial facilities in Canada have been reduced from 54 900 kg in 2000 to 14 500 kg in 2006. No releases to water or land have been reported (NPRI 2006). Recent information gathered under CEPA 1999 through a section 71 notice with respect to isoprene included reports of releases to air in 2006 in a quantity greater than 10 000 kg (Environment Canada 2007a).

Environmental Fate

Isoprene, having a very high vapour pressure of 73 327 Pa and a low boiling point of 34°C, is expected to exist solely as a vapour in the atmosphere, where it will be degraded by reaction with photochemically-produced hydroxyl radicals, ozone molecules and nitrate radicals. The half-lives for the reaction in air with hydroxyl radicals and ozone molecules are estimated to be four and 19 hours, respectively (HSDB 2008).

If released to water, isoprene is expected to volatilize from the water surface, based on its very high Henry's Law constant. Volatilization half-lives for a model river and a model lake are estimated to be one and 78 hours, respectively (HSDB 2008). If released to soil, isoprene is expected to have moderate mobility, based upon an estimated log K_{oc} of 2.69. Volatilization from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's Law constant of 0.077 atm-cu m/mole. The estimated K_{oc} indicates that isoprene is not expected to adsorb to suspended solids and sediment (HSDB 2008).

Persistence and Bioaccumulation Potential

Environmental Persistence

Once released into the environment, isoprene is likely to degrade significantly in all environmental compartments. Experimental half-life values of 0.11 days for photodegradation and 0.8 days for ozone reaction in air (Atkinson 1989) indicate that isoprene degrades relatively rapidly; therefore, the substance does not meet the persistence criterion for air (half-life of ≥ 2 days) set out in the *Persistence and Bioaccumulation Regulations*. (Canada 2000a).

A quantitative structure-activity relationship (QSAR)-based weight of evidence approach (Environment Canada 2007b) was applied to predict the persistence in water (Table 3). Based on the predicted half-lives and biodegradation value in water, it can be concluded that isoprene does not persist in water (half-life ≥ 182 days). The predicted biodegradation half-life of 15 days in water was further used to predict the half-life of this chemical in soil and sediment by applying Boethling's extrapolation factors ($t_{1/2water}$: $t_{1/2\ soil}$: $t_{1/2\ sediment}$ = 1: 1:4) (Boethling et al. 1995). According to these values, it is

concluded that this chemical does not meet the persistence criteria for water and soil (half-life ≥ 182 days) or sediment (half-life ≥ 365 days) set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000a).

Table 3. Modelled data for persistence of isoprene in water

Medium	Fate process	Degradation value	Degradation endpoint	Reference
Water	Biodegradation	15	Half-life (days)	BIOWIN 2000 Ultimate survey
Water	Biodegradation	0.67	Probability	BIOWIN 2000, MITI Non-linear Probability
Water	Biodegradation	0.54	Probability	BIOWIN 2000, MITI Linear Probability
Water	Hydrolysis	n/a[1]	Half-life (days)	HYDROWIN 2000

[1] A hydrolysis rate could not be estimated for this type of compound; consequently, hydrolysis was not considered in the determination of persistence for this compound.

Potential for Bioaccumulation

Experimental and modelled log K_{ow} values of 2.42 and 2.58, respectively, indicate that the potential for bioaccumulation is likely to be low. Experimental bioconcentration (BCF) values for fish range from 9.5 to 12.9 L/kg (MITI 1992) and modelled predictions for bioaccumulation and bioconcentration factors range from 6.3 L/kg to 117 L/kg (Table 4). Therefore, isoprene does not meet the bioaccumulation criterion (BCF, BAF ≥ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000a).

Table 4. Modelled data for bioaccumulation of isoprene

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BAF[1]	6.3 L/kg	Gobas BAF T2MTL (Arnot and Gobas 2003)
Fish	BCF[2]	5.9 L/Kg	Gobas BCF T2LTL (Arnot and Gobas 2003)
Fish	BCF	117 L/kg	OASIS Forecast 2005
Fish	BCF	14.6 L/kg	BCFWIN 2000

[1] Bioaccumulation factor
[2] Bioconcentration factor

Potential to Cause Ecological Harm

As indicated earlier, isoprene does not meet the criteria for persistence in air, water, soil or sediment, nor does it meet the criterion for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000a).

Experimental ecotoxicological data (ECOTOX database) indicate that isoprene does not cause significant harm to aquatic organisms at low concentrations. For four species of fish, acute LC_{50} values vary within a range of 42.54 to 240 mg/L.

The NPRI reports the releases of isoprene to air from industrial facilities in Canada have been reduced from 54 900 kg in 2000 to 14 500 kg in 2006. No releases to water or land have been reported (NPRI 2006). In recent information gathered through a section 71 notice under CEPA 1999 (Environment Canada 2007a), companies reported the release of isoprene in 2006 in a quantity greater than 10 000 kg, exclusively to air. Given the quantity and nature of these releases, they are deemed unlikely to result in significant exposure of organisms in the environment.

Based on the information available, the conclusion has been reached that it is unlikely that isoprene is causing ecological harm in Canada.

Potential to Cause Harm to Human Health

Exposure Assessment

Appendix 1 presents the upper bounding estimates of intake of isoprene for each age group in the general population of Canada, based on the maximum concentrations of the substance measured in indoor and outdoor air and data for one type of beverage. These upper bounding estimates of intake range from 5.6 μg/kg-bw per day (seniors, 60+ years) to 16.8 μg/kg-bw per day (children, 0.5–4 years). These estimates indicate that indoor air is the most important source of environmental exposure to isoprene for all age groups of the general population of Canada, typically comprising approximately 95% of total exposure. Ambient air is the next highest source of exposure, while only limited data for beverages were available to estimate intake from food. No data on concentrations of isoprene in drinking water and soil were identified, although contribution from these media is expected to be negligible in comparison to that from air, based on information on the physical/chemical properties, use patterns and releases of the substance.

Isoprene is the predominant unsaturated hydrocarbon present in sidestream cigarette smoke, and average yields of 3100 µg/cigarette have been measured in a test chamber (Lofroth et al. 1989). Health Canada, which has been monitoring tobacco smoke of cigarettes sold in Canada for over 30 years, reports that the emission data of cigarettes sold in Canada in 2004 show that the level of isoprene in Canadian cigarette smoke under ISO standard smoking conditions in mainstream smoke is 30-397 µg/cigarette and 395-864 µg/cigarette under modified smoking conditions. Furthermore, the level of isoprene found in sidestream smoke is 90-3194 µg/cigarette (as per email from Tobacco Control Directorate, July 29, 2008). In another study, it has been reported that isoprene comprised 16.7% of C2–C8 hydrocarbons present in the indoor air of a smoky café in Sweden (Barrefors and Petersson 1993). Also, in a monitoring study conducted in homes and workplaces in the Philadelphia, PA area, mean concentrations of isoprene in personal air samples ranged from 4.65 µg/m^3 in non-smoking homes to 18.2 µg/m^3 in homes with

smokers, and from 5.29 μg/m^3 in non-smoking workplaces to 22.8 μg/m^3 in workplaces with smokers. The approximate four-fold increases in levels of isoprene in environments where smoking occurs compared to environments without smoking were considered highly significant (Heavner et al. 1996). Thus, smoking is expected to be the primary source of isoprene in the indoor air of homes and other environments where smokers are present.

Consumer products containing polymer derived from polyisoprene such as paint resins, footwear, adhesives, moulded goods and motor oil viscosity improvers may contain residual levels of unreacted isoprene and contribute to levels in indoor air as well as to consumer exposure. For example, 17 of 19 samples of polyisoprene analyzed for residual monomer had no detectable levels of isoprene (detection limit 0.02 ppm) while the remaining two samples contained amounts that ranged between 0.02 and 0.04 ppm (OECD 2005). No attempt was made to quantify exposure from this source because of the lack of adequate information needed to complete an exposure estimate (e.g., release rate of unreacted monomer from polyisoprene). However, based on the very low levels identified, off-gassing of isoprene from polyisoprene products would likely be only a minor contributor to overall indoor air levels. Furthermore, because of the very high vapour pressure and low log K_{ow} for isoprene, emissions are expected to partition directly to air and dermal uptake of isoprene from handling polyisoprene products would likely be negligible.

Isoprene is endogenously produced in the human body, possibly as a by-product of isoprenoid biosynthesis or as an end product of isoprenoid degradation, typically comprising from 30-70% of the total hydrocarbons exhaled, with an estimated quantity exhaled ranging from 2–4 mg/day. This quantity did not appear to vary with age, sex, ethnicity, diet, life style or fasting/non-fasting state (Gelmont et al.1981). Furthermore, based on extrapolations from data in mice and rats, it has been reported that the average quantities of endogenously produced isoprene formed in the body ranged from 264–332 pmol/ml of tissue (Hartmann 1994). Several other studies also report on the levels of endogenously produced isoprene found in humans, including a range of 15–70 nmol/L (1.0–4.8 μg/L) in blood (Cailleux et al. 1992) and a production rate of 0.15 μmol/kg/h (about 17 mg/day for a 70 kg adult) (Taalman 1996). In another study, it was clearly shown that levels of isoprene in classrooms were much higher following occupation by children than background levels in unoccupied classrooms, and the authors concluded that the substance can be regarded as a measureable index of contaminants from human origin (Cailleux et al. 1993). Thus, endogenous production of isoprene may contribute to observed indoor air levels of the substance. However, it was also reported that while isoprene is formed endogenously, 90% will be metabolized within the body and only about 10% exhaled unchanged (Filser et al. 1996). Phillips et al (1994) reported that concentrations of isoprene were greater in alveolar air than in inspired air, consistent with an exogenous origin and uptake and catabolism in vivo or excretion via an extrapulmonary pathway.

Confidence in the upper bounding estimate of intake of isoprene through environmental media is considered to be high, since recent Canadian monitoring data were available for

the most relevant media of exposure (i.e., indoor and ambient air). Although no data were available for drinking water and soil and only limited data for food, it is expected that these media are not major sources of exposure.

Health Effects Assessment

An overview of the toxicological database for isoprene is presented in Appendix 2.

On the basis of investigations in experimental animals, isoprene has been classified by the International Agency for Research on Cancer (IARC) as Group 2B (*Possibly carcinogenic to humans*) (IARC 1999) and by the European Commission as Category 2 (*Regarded as if they are carcinogenic to man; May cause cancer*) (ESIS [date unknown]; European Commission 2004). As well, the U.S. National Toxicology Program (NTP) has classified isoprene as being ". . . *reasonably anticipated to be a human carcinogen . . .*" (NTP 2004). These classifications were based on increased incidences of neoplastic effects at multiple sites in both mice and rats exposed to isoprene via inhalation, as described below.

In a stop-exposure bioassay in male B6C3F_1 mice exposed via inhalation to up to 19 530 mg/m^3 of isoprene for 26 weeks and observed for an additional 26 weeks, increased incidences of tumours in several tissues were observed, including alveolar/bronchiolar adenoma or carcinoma, Harderian gland adenoma, hepatocellular adenoma or carcinoma and forestomach squamous-cell papilloma or carcinoma (NTP 1995, Melnick et al. 1994). In an additional study in B6C3F_1 mice, males were exposed via inhalation to concentrations of up to 6138 mg/m^3 of isoprene for 20, 40 or 80 weeks and were observed until 96 or 104 weeks, while female mice were exposed via inhalation to concentrations of up to 195 mg/m^3 for 80 weeks and observed until 104 weeks. Male mice had significantly increased incidences of tumours of the lung, liver, and Harderian gland in addition to histiocytic sarcomas. As well, non-significantly increased incidences of heart and spleen haemangiosarcomas were observed in males. Increased incidences of Harderian gland and pituitary adenomas (significant) were observed in female mice exposed to the highest concentration (Placke et al. 1996).

Male and female F344/N rats were exposed to concentrations of up to 19 530 mg/m^3 of isoprene for 104 weeks. There were increased incidences of mammary fibroadenoma in males and females, as well as renal tubule adenomas or carcinomas and interstitial-cell adenoma of the testis in males (NTP 1999a). A slight increase in the incidence of interstitial-cell adenoma of the testis was also observed in male F344/N rats in a stop exposure study, where animals were exposed via inhalation to concentrations up to 19 530 mg/m^3 of isoprene for 26 weeks, and were observed for a further 26 weeks (Melnick et al. 1994, 1996; NTP 1995). However, an IARC Working Group noted that spontaneous incidence of this tumour type is high in two-year studies, and that the duration was not adequate for evaluation of carcinogenic potential (NTP 1994a; IARC 1994), although it is noteworthy that the incidence increased after only 26 weeks of exposure.

The European Commission has also classified isoprene as Category 3 regarding its mutagenicity (*Substances which cause concern for man owing to possible mutagenic effects; Possible risk of irreversible effects*) (ESIS [date unknown]; European Commission 2004). Isoprene was genotoxic in *in vivo* assays as positive results were observed for sister chromatid exchanges and micronuclei induction in bone-marrow cells in mice exposed via inhalation (IARC 1999). However, negative results were observed in some assays conducted *in vitro*, specifically tests for mutations in bacteria, and sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells (IARC 1999). A positive result was observed for the Comet assay in blood cells *in vitro* (Fabiani et al. 2007). According to IARC (1994), the genotoxic activity of isoprene *in vivo* may be due to a metabolite of isoprene, 2-methyl-1,2:3,4-diepoxybutane.

Although thorough analyses of the potential mode of action for induction of tumours by isoprene is considered to be beyond the scope of this screening assessment, the NTP has postulated that reactive alkylating metabolites of isoprene may be involved, but it is also noted that other mechanisms should be considered (NTP 1999b).

Exposure to isoprene via inhalation, the predominant route of exposure for the general population, also induced a variety of non-cancer effects in experimental animals, often at the lowest concentration tested. In addition, hyperplasia occurred at sites at which tumours were also observed (lungs, forestomach, kidneys and testis). The lowest lowest-observed-effect-concentration (LOEC) for exposure to isoprene is 11 mg/m^3, which was associated with an increase in proliferative activity of the thymus of Wistar rats exposed for four months, followed by one month of observation (Mamedov 1979). At a higher concentration, a variety of effects such as changes in thymus weight, mitotic index and cellularity of the thymus were observed to be reversible during exposure or recovery, but at the lower concentration, the increased proliferation of the thymus was noted during the recovery period. As well, a short-term LOEC of 98 mg/m^3 was identified for increased proliferative activity in the thymus in male Wistar rats exposed for 30 days (Mamedov 1979). As in the subchronic study, reversible changes were observed in the thymus as well as in the spleen, but the proliferative changes remained at the end of the study. Although both studies are limited in the scope of organs and biological systems investigated, in other studies which involved more comprehensive gross and histopathology analyses, effects were also noted in the thymus (change in thymus weight, thymic atrophy) as well as the spleen (change in spleen weight) of rats and mice exposed to higher concentrations of isoprene for 2, 13 or 26 weeks (NTP 1995). In addition, splenic fibrosis was observed in rats exposed for 105 weeks to 1953 mg/m^3 and above (NTP 1999a). The next lowest LOEC of approximately 28 mg/m^3 was identified for a non-significant decrease in ovarian weight in female mice exposed to up to 195 mg/m^3 of isoprene for 80 weeks (Placke et al. 1996). (It is noteworthy that ovarian atrophy was induced at low concentrations in mice after exposure to 1,3-butadiene, a structural analogue of isoprene [NTP 1984, 1993; Bevan et al. 1996].) Additional effects observed on the female reproductive system, including increased estrous cycle length and decreased gravid uterine weight were observed in female mice exposed to higher concentrations for shorter durations (Melnick et al. 1994; NTP 1995).

The confidence in the toxicity database in experimental animals is considered to be moderate to high, as data were identified for acute, repeat-dose, reproductive and developmental toxicity, carcinogencity and genotoxicity; however, the level of detail reported in some of the repeat dose studies is limited and no sufficient human epidemiology data were available. There is also uncertainty regarding the mode of induction of tumours.

Characterization of Risk to Human Health

Based principally on the weight of evidence based assessments of several international and national agencies (IARC 1999, NTP 2004, NTP 1999b, European Commission 2004), a critical effect for characterization of risk to human health for isoprene is carcinogenicity. In chronic bioassays, isoprene consistently induced tumours at multiple sites in both mice and rats. This substance was also genotoxic in *in vivo* assays in mice. Thus, in light of these data, a mode of induction for tumours involving direct interaction with genetic material cannot be precluded. Although epidemiological data are inadequate for evaluation, isoprene is a structural analogue of 1,3-butadiene, which has been associated in lymphohaematopoietic cancer in exposed workers.

With respect to consideration of critical non-cancer effects in a screening context, comparison of the conservatively selected lowest identified inhalation effect level (11 mg/m^3 or 11 000 $\mu g/m^3$) from a subchronic study, with the highest identified concentration of isoprene (30.5 $\mu g/m^3$) reported in indoor air in Canada in 2006, the principal source of exposure for the general population, results in a margin of exposure of approximately 360. Comparison of this effect level with the highest identified concentration of 9.48 $\mu g/m^3$ reported in ambient air in an urban area in Canada yields a margin of exposure of approximately 1160. While it is recognized that endogenous production and natural sources of isoprene contribute to overall exposure in addition to anthropogenic sources, it is not possible to separate the relative contribution of each of these sources in the scope of a screening level assessment. In addition, data indicate that cigarette smoking results in higher concentrations of isoprene in indoor air; therefore, the margin of exposure may be smaller in the homes of smokers. Thus, in light of the uncertainties in the databases, including those relating to the mode of induction of tumours, it is considered that these margins of exposure may not be adequately protective of human health.

Uncertainties in Evaluation of Risk to Human Health

The scope of this screening assessment of isoprene does not take into account variability across the general population or differences between humans and experimental animals with respect to differences in sensitivity of induction of effects, particularly in light of the lack of sufficient epidemiological studies. However, the metabolic pathway of isoprene is qualitatively similar between experimental animals and humans, although there may be slight quantitative differences (Gervasi and Longo 1990; Csanady and Filser 2001, Peter et al. 1987). In addition, the available data support a similar profile of health effects as

that for 1,3-butadiene, which has been associated with cancer in humans. Although isoprene induced tumours at multiple sites in multiple experimental animal species, the method of tumour induction has not been fully elucidated. However, there are indications that metabolites of the substance could play a role, potentially through interaction with genetic material. In addition, the critical study for non-cancer effects was limited in the range of organs and biological systems investigated.

There are also significant uncertainties regarding the relative contribution of biogenic sources of isoprene, such as trees and other plants, and endogenous production of the substance in humans. While it is not possible to distinguish between the relative contributions of exogenous and endogenous sources of isoprene reaching target tissues, exposure to exogenous isoprene induced tumours in rodents, which also produce the substance endogenously. However, there is considerable variation in reported rates of endogenous production for different species. Quantitative comparison of kinetics and dynamics of isoprene between humans and experimental species could elucidate differences in sensitivities to external sources in terms of tumourgenicity, but such analyses are beyond the scope of this screening assessment.

In addition, the contribution of emissions from consumer products manufactured from polyisoprene, butyl rubber and SIS rubber to population exposure to isoprene is unknown, although likely to be insignificant in comparison to other anthropogenic or natural sources.

Conclusion

Based on the available information, it is concluded that isoprene is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity, or that constitute or may constitute a danger to the environment on which life depends. Additionally, isoprene does not meet the criterion for persistence or for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations*.

On the basis of the carcinogenicity of isoprene, for which there may be a probability of harm at any level of exposure, as well as the potential inadequacy of the margins of exposure for non-cancer effects, it is concluded that isoprene be considered as a substance that may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that isoprene does not meet the criteria in paragraph 64a and 64b of CEPA 1999, but that it does meet the criteria in paragraph 64c of CEPA 1999.

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Appendix 1: Upper Bounding Estimates of Daily Intake of Isoprene by the General Population in Canada

Estimated intake (µg/kg b.w.-day) of isoprene by various age groups								
Age group	**0–6 months[1,2,3]**			**0.5–4 yr[4]**	**5–11 yr[5]**	**12–19 yr[6]**	**20–59 yr[7]**	**60 + yr[8]**
Route of exposure	**Breast fed**	**Formula fed**	**Not formula fed**					
Ambient air[9]	0.33			0.71	0.55	0.32	0.27	0.24
Indoor air[10]	7.5			16.0	12.5	7.1	6.1	5.30
Drinking water[11]	na[12]	na	na	na	na	na	na	na
Food and beverages[13]			na	na	na	0.03	0.06	0.04
Soil[14]	na			na	na	na	na	na
Total intake	7.8	7.8	7.8	16.8	13.1	7.5	6.4	5.6

1 No measured data were identified on the concentration of isoprene in breast milk.

2 Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, drink 0.8 L/day of water (formula fed) or 0.3 L/day (not formula fed) or 0.74 L/day of breast milk and ingest 30 mg of soil per day (Health Canada 1998). Breast-fed and formula-fed infants are assumed to consume no other foods.

3 For exclusively formula-fed infants, intake from water is synonymous with intake from food. The concentration of isoprene in water used to reconstitute formula was based on available water data. No data on concentrations of isoprene in formula were identified for Canada. Approximately 50% of infants are introduced to solid foods by four months of age and 90% by six months of age (NHW, 1990 [cited in Health Canada 1998]).

4 Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, drink 0.7 L/day of water and ingest 100 mg/day of soil (Health Canada 1998).

5 Assumed to weigh 31.0 kg, to breathe 14.5 m^3/day of air, drink 1.1 L/day of water and ingest 65 mg/day of soil (Health Canada, 1998).

6 Assumed to weigh 59.4 kg, to breathe 15.8 m^3/day of air, to drink 1.2 L/day of water and ingest 30 mg/day of soil (Health Canada 1998).

7 Assumed to weigh 70.9 kg, to breathe 16.2 m^3/day of air, drink 1.5L/day of water and ingest 30 mg/day of soil (Health Canada 1998).

[8] Assumed to weigh 72.0 kg, to breathe 14.3 m^3/day of air, drink 1.6 L/day of water and ingest 30 mg/day of soil (Health Canada 1998).

[9] The highest concentration of isoprene (9.48 μg/m^3) identified in ambient air samples collected from one of 15 sites across Canada (Environment Canada 2006) was used to calculate the upper bounding estimate of exposure. It is assumed that Canadians spend 3 hours/day outdoors (Health Canada 1998). The critical data were selected from a dataset of Canadian studies of ambient air (Health Canada 2008).

[10] The highest concentration of isoprene (30.5 μg/m^3) identified in indoor air samples collected from randomly selected non-smoking homes in Windsor, Ontario (Health Canada 2008) was used to calculate the upper bounding estimate of exposure. It is assumed that Canadians spend 21 hours/day indoors (Health Canada 1998).

[11] No reported concentrations of isoprene in tap water in Canada or elsewhere were identified.

[12] Not available

[13] The only available information on concentration of isoprene in food and beverages was a single study from Germany showing that it it was present at 4 μg/kg in beer (Bohmann, 1984). This value was used to calculate isoprene intake from food and beverages for the relevant age groups (12-19 yrs, 20-59 yrs, 60+ yrs). However, isoprene may be used to manufacture an isobutylene-isoprene copolymer (butyl rubber) that is used a chewing gum base, and it is possible that residual amounts of the monomers may be ingested while chewing gum. While the maximum allowable level of isoprene in the copolymer is 10 ppm (Lanxess, 2005), recent surveys by the industry failed to detect the presence of either isoprene or isobutylene in samples of gum base or finished chewing gum using a detection limit of 1 ppm. The non-detection is probably due to volatization and dilution of the unreacted monomers during the production of gum base and finished chewing gum (as per email from Health Products and Food Branch, June 20 2008). Thus, the contribution to the overall intake of isoprene from this potential source will likely be insignificant.

[14] No reported concentrations were identified of isoprene in soil in Canada or elsewhere.

Appendix 2: Summary of Health Effects Information for Isoprene

Endpoint	Lowest effect levels[a]/Results
Acute toxicity	**Lowest oral LD_{50}** = 2043–2210 mg/kg in rats (Kimmerle and Solmecke 1972) Additional studies: N/A **Lowest inhalation LC_{50}** = 139 000 mg/m^3 in male mice (Gostinskii 1965) Additional studies: Shugaev 1969; Mamedov 1979; Korbakova and Fedorova 1964; LC_{50} values expressed as '"greater than": Kimmerle and Solmecke 1972; Bayer 1972; Rhone-Poulenc, Inc. 1971 **Lowest inhalation LO(A)EC** = 20 ppm or 46 mg/m^3 in $B6C3F_1$ mice after a 6-hour exposure for respiratory effects such as depressed breathing frequency (0, 46, 460, 4600 mg/m^3) (Bond et al. 1991) Additional studies: Mamedov 1979; Korbakova and Fedorova 1964; Von Oettingen 1940; Gostinskii 1965; Rohr et al. 2002; Wilkins et al. 2001; Dahl et al. 1987 **Lowest dermal LD_{50}** > 681 mg/kg bw in rats (Bayer 1972) Additional studies: Kimmerle and Solmecke 1972
Short-term repeated-dose toxicity	**Lowest oral LOEL** = N/A Lowest oral NOEL = 200 mg/kg body weight in male rats where no effects were noted; the dose started at 200 mg/kg body weight and increased by a factor of 1.5 over the following 4 days; animals were observed for an additional 7 days (Bayer 1972) Additional studies: Del Monte et al. 1985 **Lowest inhalation LOEC** = 0.098 mg/L or 98 mg/m^3 (lowest concentration tested) in rats for effects observed on the thymus (increased mitotic index) after exposure by inhalation (0, 98, 1016 mg/m^3 for 4 hours per day for 30 days) (Mamedov 1979). Additional studies: LOEL = 438 ppm[b] or 1220 mg/m^3 (lowest concentration tested) for lesion formation in the liver, forestomach; changes in haematology and organ weights (liver, thymus) of mice exposed to up to 19 530 mg/m^3 for 6 hours/day for 5 days/week over 2 weeks (Melnick et al. 1990; NTP 1995); Von Oettingen 1940; Melnick et al. 1990; NTP 1995; Gage 1970; Bayer 1972
Subchronic toxicity	**Lowest inhalation LOEC** = 0.0108 mg/L or 11 mg/m^3 (lowest concentration tested) in Wistar rat exposed to up to 116 mg/m^3 for 4 months for effects observed in the thymus (increased proliferative activity) after one month of recovery (0, 11, 116 mg/m^3 for 4 hours/day for 4 months) (Mamedov 1979). Additional studies: LOEL = 70 ppm[b] or 195 mg/m^3 (lowest concentration tested) in $B6C3F_1$ mice due to spinal cord degeneration after 26 weeks of exposure and 26 weeks of recovery by inhalation at exposure levels of up to 19 530 mg/m^3 (0, 195, 614, 1953, 6138, 19 530 mg/m^3 for 6 hours/day, 5 days/week for 26 weeks with an additional 26 weeks of observation); changes in haematology, muscle strength, organ weights (liver, spleen,

Endpoint	Lowest effect levels[a]/Results
	brain) and histology (lesions in forestomach, liver, harderian gland, lungs, nose) observed at higher exposure levels in mice (Melnick et al. 1994, 1996; NTP 1995). Placke et al. 1996; Melnick et al. 1994; Gostinskii 1965 (range of levels); Faustov 1972; Samedov et al. 1978; Faustov and Lobeeva 1970 (single level studies)
Chronic toxicity/carcinogenicity	**Lowest inhalation LOEC** = 70 ppm[b] or 195 mg/m^3 for significant mild metaplasia of the olfactory epithelium in the nose of female mice exposed to up to 195 mg/m^3 for 80 weeks. Due to study exposure protocol for male mice and the reporting of results, it is difficult to select an effect level for male mice in this study (Placke et al. 1996); also LOEC = 70 ppm or 195 mg/m^3 for spinal cord degeneration in mice exposed for 26 weeks and observed until 52 weeks (NTP 1995; Melnick et al. 1994). According to the NTP, "A NOAEL was not achieved for spinal cord degeneration . . ." (NTP 1995). Additional studies: (NTP 1999a) *Bioassasys in mice* B6C3F$_1$ mice (male) were exposed by inhalation to 0, 70, 220, 700, 2200, or 7000 ppm (0, 195, 614, 1953, 6138 or 19 530 mg/m^3) for 6 hours/day, 5 days/week over 26 weeks and were monitored for an additional 26 weeks; 10 mice per group were sacrificed after cessation of exposure. A significant increase in tumours was observed at or above 1953 mg/m^3, specifically, alveolar/bronchiolar adenoma or carcinoma (0, 195, 614, 1953, 6138, 19 530 mg/m^3: 2/30, 2/30, 1/29, 5/30, 10/30, 9/28), Harderian gland adenoma (0, 195, 614, 1953, 6138, 19 530 mg/m^3: 2/30, 6/30, 4/30, 14/30, 13/30, 12/30), hepatocellular adenoma or carcinoma (0, 195, 614, 1953, 6138, 19 530 mg/m^3: 7/30, 3/30, 7/29, 15/30, 18/30, 17/28) and forestomach squamous-cell papilloma or carcinoma (0, 195, 614, 1953, 6138, 19 530 mg/m^3: 0/30, 0/30, 0/30, 1/30, 4/30, 6/30). There were also increased incidences of multiple tumours and/or neoplasms of greater malignancy in the liver of exposed mice compared to controls. The incidence of hyperplasia in the lung and forestomach was increased at 1953 mg/m^3 and above in mice sacrificed at the end of the exposure period. Significant changes in haematological parameters indicative of nonresponsive macrocytic anaemia were noted at 614 mg/m^3 and above. Spinal cord degeneration was observed in all exposed groups. (NTP 1995; Melnick et al. 1994). Male B6C3F$_1$ mice were exposed to 0, 10, 70, 140, 280, 700 or 2200 ppm (0, 28, 195, 391, 781, 1953 or 6138 mg/m^3) by inhalation for 4 or 8 hours/day, 5 days/week over 20, 40 or 80 weeks. Female B6C3F$_1$ mice were exposed to 0, 10 or 70 ppm (0, 28, 195 mg/m^3) for 80 weeks. Post-exposure observation lasted until week 96 or 104. Male mice had a significant increase in incidence of alveolar/bronchiolar adenoma and carcinoma (at 1953 mg/m^3 and above), hepatocellular adenoma and carcinoma or Harderian gland adenoma (at 391 mg/m^3 and above), as well as histiocytic sarcomas (found in kidney, lung, lymph nodes, bone marrow and spleen) (at 391 mg/m^3 and above). Alveolar and forestomach hyperplasia was noted at the higher concentrations (not specified). Non-significant increases in incidence of heart and spleen haemangiosarcoma were observed. Female mice had a significantly increased incidence of Harderian gland adenoma (0, 28, 195 mg/m^3: 2/49, 3/49, 8/49), and pituitary adenomas (0, 28, 195 mg/m^3: 1/49, 6/46, 9/49) at 195 mg/m^3. Metaplasia of the nasal cavity was noted in males at 391 mg/m^3 and in females at 195 mg/m^3. Haematopoietic cell proliferation in the spleen and myeloid hyperplasia of the bone marrow was slightly increased in all exposed groups (significance not reported). Due to study exposure protocol for male mice and the reporting of results, it is difficult to select an effect level for male mice exposed for 20, 40 and 80 weeks to levels up to 6138 mg/m^3 (Placke et al. 1996). *Bioassays in rats* F344/N rats (male and female) were exposed by inhalation to 0, 220, 700, 7000 ppm (0,

Endpoint	Lowest effect levels[a]/Results
	614, 1953 or 19 530 mg/m^3) for 6 hours/day, 5 days/week over 105 weeks. Both male and females had increased incidence of mammary fibroadenomas (male [significant at 19 530 mg/m^3, but above historical controls at 614, 1953 mg/m^3] – 0, 614, 1953, 19 530 mg/m^3: 2/50, 4/50, 6/50, 21/50; female [significant at all levels] – 0, 614, 1953, 19 530 mg/m^3: 19/50, 35/50, 32/50, 32/50). Male rats in the 1953 mg/m^3 and 19 530 mg/m^3 groups had a significant increase in the incidence of renal tubule adenoma (0, 614, 1953, 19 530 mg/m^3: 2/50, 4/50, 8/50, 15/50) and interstitial cell adenoma of the testis (0, 614, 1953, 19 530 mg/m^3: 33/50, 37/50, 44/50, 48/50). Low incidences of several brain tumours were observed in female rats, including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumour and meningeal sarcoma. These tumours are rare in control F344/N rats and were considered by the NTP (1999b) to be possibly related to isoprene exposure. Overall, the NTP (1999a) found that male rats demonstrated "clear evidence" of carcinogenic activity, while female rats demonstrated "some evidence" of carcinogenic activity. The incidence of renal tubule hyperplasia (distinguishable from age-related nephropathy) and splenic fibrosis was significantly increased in males at 1953 and 19 530 mg/m^3 (NTP 1999a). Groups of male F344/N rats were exposed via inhalation to concentrations of isoprene of 0, 70, 220, 700, 2200, or 7000 ppm (0, 195, 614, 1953, 6138 or 19 530 mg/m^3) for 6 hours/day, 5 days/week for 26 weeks, and were observed for a further 26 weeks; 10 rats exposed to the highest concentration were sacrificed after cessation of exposure. An increase was observed in the incidence of interstitial-cell adenoma (non-significant, but slightly greater) of the testis in male rats (0, 195, 614, 1953, 6138, 19 530 mg/m^3: 3/30, 3/30, 4/30, 7/30, 8/29, 9/30). Interstitial cell hyperplasia was observed in the testes of rats examined at the end of the exposure period. (Melnick et al. 1994, 1996; NTP 1995). An International Agency for Research on Cancer Working Group noted that spontaneous incidence of this tumour type is high in two-year studies, and that the duration was not adequate for cancer evaluation (IARC 1994) It was also noted that the 26-week study in F344/N rats provided no conclusion regarding carcinogenic activity (NTP 1999a)
Reproductive toxicity	No studies conducted with isoprene specifically designed for reproductive endpoints. **Lowest inhalation LOEC** = 10 ppm or 28 mg/m^3 (lowest concentration tested) in female mice for ovarian effects (non-significantly reduced ovarian weight) observed after 80 weeks of exposure to up to 195 mg/m^3 in female B6C3F$_1$ mice (Placke et al. 1996). (N.B.: No data provided regarding incidence of effect.) Additional studies: Repina 1988; Melnick et al. 1994; NTP 1995;Doerr et al. 1995 (intraperitoneal injection) shows effects in ovarian follicles of mice.

Endpoint	Lowest effect levels[a]/Results
Developmental toxicity	**Lowest fetal inhalation LOEC =** 280 ppm or 781 mg/m^3 (lowest concentration tested) in Swiss mice due to decreased fetal body weight in female pups, where maternal mice were exposed to isoprene concentrations up to 19 530 mg/m^3 for 6 hours/day, 7 days/week over gestational days 6–17 (Mast, Evanoff et al. 1989; Mast, Rommereim et al. 1990; NTP 1989, 1995). (According to the NTP, "A NOAEL was not achieved for . . . developmental toxicity . . " [NTP 1995].) **Lowest maternal inhalation LOEC =** 7000 ppm or 19 530 mg/m^3 in Swiss mice due to a significant reduction in uterine weight and maternal body weight (Mast, Evanoff et al. 1989; Mast, Rommereim et al. 1990; NTP 1989; NTP 1995) Additional studies: Mast, Evanoff et al. 1989; Mast, Rommereim et al. 1990; NTP 1989, 1995 **Lowest oral LOEL =** 1895 mg/kg body weight as no embryotoxicity or teratogenicity observed after treatment of Wistar rat on gd 9-12; although slightly reduced ossification of the sternebrae and occipital bone observed in fetus (Tsutsumi et al. 1969).
Genotoxicity and related endpoints: *in vivo*	**Chromosomal aberrations** Negative in bone marrow of mice exposed via inhalation (Tice et al. 1988) **Micronuclei test** Positive in bone marrow of mice exposed via inhalation (Tice et al. 1988) Positive in peripheral blood of mice exposed by inhalation (Placke et al. 1996) Negative in lung fibroblasts of rats exposed via inhalation (NTP 1999a) **Sister Chromatid Exchange** Positive in bone marrow of mice exposed by inhalation (Tice et al. 1988) **Covalent Binding/Adduct formation** Positive in hemoglobin of mice exposed by inhalation (Bond et al. 1991) Positive in hemoglobin of mice and rats exposed by a single intraperitoneal injection (Sun et al. 1989) Increase in levels of hemoglobin adducts formed in mice and rats after intraperitoneal injection (Tareke et al. 1998) Additional study: NTP 1994b (no details reported in BG Chemie [2000] regarding hemoglobin adduct quantity formed)
Genotoxicity and related endpoints: *in vitro*	**Chromosomal aberrations** Negative in Chinese hamster ovary cells, with and without activation (NTP 1995; Galloway et al. 1987) **Mutagenicity** Negative for reverse mutations in *S. typhimurium* TA100, TA1530, TA1535, TA1538, TA98, TA1537, *E. coli* WP2uvrA/pkM101, with and without activation (de Meester et al. 1981; Mortelmans et al. 1986; Madhusree et al. 2002) Negative for reverse mutations in *S. typhimurium* TA102, TA104 without activation (Kushi et al. 1985) Negative for reverse mutations in *S. typhimuruium* TA1535, TA1537, TA98, TA100, *E. coli* WP2uvrA with activation (Huntingdon Life Sciences 2003) **Comet Assay**

Endpoint	Lowest effect levels[a]/Results
	Positive in peripheral blood mononuclear cells with activation (Fabiani et al. 2007). Negative without activation in peripheral blood mononuclear cells; negative with and without activation in human promyelocytic leukemia cells (HL60) (Fabiani et al. 2007). **Sister chromatid exchange** Negative in Chinese hamster ovary cells with and without activation (NTP 1995; Galloway et al. 1987)

[a] LD_{50} = median lethal dose; LC_{50} = median lethal concentration; LO(A)EL = Lowest-observed –(adverse)-effect level; LO(A)EC = Lowest-observed-(adverse)-effect concentration; NO(A)EL = No-observed-(adverse)-effect level; NO(A)EC = No-observed-(adverse)-effect concentration

[b] Conversion factor: 1 ppm = 2.79 mg/m^3 at 25ºC (IARC 1999)