Screening Assessment for the Challenge

2-Cyclohexen-1-one, 3,5,5-trimethyl-

(Isophorone)

Chemical Abstracts Service Registry Number
78-59-1

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 2-cyclohexen-1-one, 3,5,5-trimethyl- (isophorone), Chemical Abstracts Service Registry Number 78-59-1. The substance isophorone was identified in the categorization of the Domestic Substances List as a high priority for action under the Ministerial Challenge. Isophorone was identified as a high priority as it was classified by the European Commission and the US Environmental Protection Agency on the basis of carcinogenicity. The substance did not meet the ecological categorization criteria for persistence, bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, the focus of this assessment of isophorone relates primarily to human health risks.

According to information submitted under section 71 of CEPA 1999, isophorone was not manufactured by any company in Canada in the calendar year 2006. However, approximately 10 000–100 000 kg of the substance was imported in 2006, and approximately 1 000–10 000 kg was reported to be released to the atmosphere in the same year. The major use of isophorone is industrial. However, it was determined that, for the general population of Canada, the highest estimated exposure to isophorone would result from the use of isophorone as a food flavour.

Isophorone is used as a solvent for automotive and industrial coatings, including industrial metal coatings and food packaging, and in adhesives for plastics, polyvinyl chloride and polystyrene materials. Isophorone is used as a formulant in one registered pest control product in Canada, but the registration of this product was discontinued December 31, 2009.

As isophorone was classified on the basis of carcinogenicity by other national and international agencies, carcinogenicity was a key focus for this screening assessment. In long-term studies, rats showed some evidence of increased incidences of renal tubular cell adenomas and adenocarcinomas and of preputial gland carcinomas, whereas mice showed equivocal evidence of increased incidences of hepatocellular adenomas or carcinomas and of mesenchymal tumours in the integumentary system. However, the renal tumours induced by isophorone in rats involved a species-specific mechanism, the preputial gland tumours in rats were observed only at higher doses and the tumour incidence in mice were significantly increased only at higher doses. Consideration of the available information regarding genotoxicity and the conclusions of other agencies indicate that isophorone is not likely to be genotoxic. Accordingly, although the mode of induction of tumours is not fully elucidated, the tumours observed are not considered to have resulted from direct interaction with genetic material. Therefore, a threshold approach is used to assess risk to human health.

Non-neoplastic effects were observed in the kidneys of rats and in the liver of mice orally exposed to isophorone in repeated-dose studies. The margin between the highest upper-bounding estimate of exposure from food and beverages and critical effect levels is

considered to be adequately protective to account for data gaps and uncertainties in the human health risk assessment for both cancer and non-cancer effects. Based on the available information on the potential to cause harm to human health and the resulting margin of exposure, it is concluded that isophorone is a substance that is not 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 the low ecological hazard posed by isophorone, as well as information on releases of isophorone to the environment, it is concluded that the 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. Isophorone does not meet the criteria for persistence or bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations.*

It is therefore concluded that isophorone does not meet any of the criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

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 to human health.

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 inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; 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), which 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 2-cyclohexen-1-one, 3,5,5-trimethyl- (isophorone) was identified as a high priority for screening assessment of human health risk because it was considered to present GPE and had been classified by other agencies on the basis of carcinogenicity. The Challenge for this substance was published in the *Canada Gazette* on August 30, 2008 (Canada 2008). 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 pertaining to the substance were received.

Although isophorone was determined to be a high priority for assessment with respect to human health, it did not meet the criteria for persistence, bioaccumulation or inherent toxicity to aquatic organisms.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution.

This final 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 March 2009 for human health and ecological sections of the document. 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) for the general population, as well as information on health hazards (based principally on the weight of evidence 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 final 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 conclusion is based.

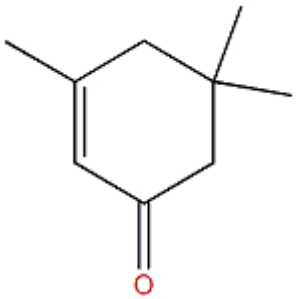
This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review/consultation. 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 Ms. Joan Strawson (TERA), Dr. Pam Williams (E Risk Sciences) and Dr. Harlee Strauss (Strauss Associates). Additionally, the draft of this screening assessment was subject to a 60-day public comment period. 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.

The critical information and considerations upon which the final assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as isophorone, based on the Philippine Inventory of Chemicals and Chemical Substances name. Information on the identity of isophorone is summarized in Table 1.

Table 1. Substance identity for isophorone

CAS RN	78-59-1
DSL name	2-Cyclohexen-1-one, 3,5,5-trimethyl-
NCI names	Cyclohex-2-en-1-one, 3,5,5-trimethyl- (PICCS) 2-Cyclohexen-1-one, 3,5,5-trimethyl- (AICS, ASIA-PAC, DSL, PICCS, SWISS, TSCA) Isophorone (PICCS) 1,5,5-Trimethylcyclohexen-3-one (ENCS) 3,5,5-Trimethyl-2-cyclohexen-1-one (ENCS) 3,5,5-Trimethyl-2-cyclohexene-1-one (ECL, PICCS) 3,5,5-Trimethylcyclohex-2-enone (DSL, EINECS)
Other names	1-Cyclohexen-3-one, 1,5,5-trimethyl- Isoacetophorone Isoforon Isophoron α-Isophoron α-Isophorone NSC 403657 NSC 4881 1,1,3-Trimethyl-3-cyclohexene-5-one 3,5,5-Trimethyl-2-cyclohexenone 1,5,5-Trimethyl-3-oxocyclohexene
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Ketones
Major chemical subclass	Cyclic ketones
Chemical formula	$C_9H_{14}O$
Chemical structure	
SMILES	O=C(C=C(CC1(C)C)C)C1
Molecular mass	138.21 g/mol

Abbreviations: AICS, Australian Inventory of Chemical Substances; ASIA-PAC, Asia-Pacific Substances Lists; CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; ECL, Korean Existing Chemicals List; EINECS, European Inventory of Existing Commercial Chemical Substances; ENCS, Japanese Existing and New Chemical Substances; NCI, National Chemical Inventories;

NZIoC, New Zealand Inventory of Chemicals; PICCS, Philippine Inventory of Chemicals and Chemical Substances; SMILES, simplified molecular input line entry specification; SWISS, Swiss Giftliste 1 and Inventory of Notified New Substances; TSCA, *Toxic Substances Control Act* Chemical Substance Inventory.
Source: NCI 2006

Physical and Chemical Properties

Table 2 contains experimental and modelled physical and chemical properties of isophorone that are relevant to its environmental fate. When available, acceptable experimental data for a property are used in preference to data generated through modelling.

Table 2. Physical and chemical properties of isophorone

Property	Type	Value[2]	Temperature (°C)	Reference
Melting point (ºC)	Experimental	−8.10		Braithwaite 1995
Boiling point (ºC)	Experimental	215.3		Braithwaite 1995
Density (kg/m^3)	Experimental	920		IPCS 1995a
Vapour pressure (Pa)	Experimental	40[3] (0.4 hPa)		Hüls AG 1981
		50.66 (0.38 mmHg)	20	Verschueren 1983
		53.33 (0.40 mmHg)		Perry and Green 1984
		58.66 (0.44 mmHg)	25	Daubert and Danner 1989
		66.66 (0.50 mmHg)	25	ISHOW 1992
		133 (1.00 mmHg)	38.0	Sax 1984
Henry's Law constant ($Pa·m^3/mol$)	Estimated[1]	6.72×10^{-1} (6.64×10^{-6} $atm·m^3/mol$)		EPIsuite 2007
Log K_{ow} (dimensionless)	Experimental	1.67		Veith et al. 1980
Log K_{oc} (dimensionless)	Modelled	1.766		PCKOCWIN 2000
Water solubility (mg/L)	Experimental	1.45×10^4 [3] (14.5 g/L)	20	Veith et al. 1980
		1.20×10^4	15–25	Parrish 1983
		1.26×10^4		ISHOW 1992

Abbreviations: K_{ow}, octanol–water partition coefficient; K_{oc}, organic carbon–water partition coefficient.
[1] Using indicated vapour pressure and water solubility.

[2] Values in parentheses represent the original values reported by the authors.
[3] Value used for fate modelling. Experimentally obtained values were selected from studies chosen by an internationally recognized organization which reviewed and identified the studies as critical studies in their own assessment.

Sources

Isophorone enters the environment through anthropogenic activities and to a lesser extent from naturally occurring sources.

Based on information submitted under section 71 of CEPA 1999, isophorone was not manufactured in Canada in 2006 above the reporting threshold of 100 kg. The total quantity imported into Canada in the same calendar year was 10 000–100 000 kg (Environment Canada 2008). According to Statistics Canada (2009), global importation of isophorone into Canada in the calendar year 2000 was 81 422 kg, which decreased to around 20 000 kg by 2003. From 2003, the global importation of isophorone into Canada remained steady at around 20 000 kg (Statistics Canada 2009).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) noted that natural occurrences of isophorone in food have been reported (JECFA 2002). Isophorone is thought to occur naturally in several types of European honey (Guyot et al. 1999; Jerkovic et al. 2006; Alissandrakis et al. 2007; Castro-Vázquez et al. 2007; de la Fuente et al. 2007), but its presence in honey sold in Canada has not been established.

Isophorone has also been identified in the combustion products of a coal-burning generating station (Harrison et al. 1985).

Uses

Based on the available scientific and technical literature, isophorone is used globally as a solvent for coating systems and as a component in metal paints and adhesives for plastics, polyvinyl chloride and polystyrene materials (ATSDR 1989).

According to information collected under section 71 of CEPA 1999, isophorone is used in industrial settings in Canada, but not in consumer products (Environment Canada 2008). Industrial applications involve a drying or curing step that reduces the amount of isophorone to very low residual levels; therefore, exposure of the Canadian population to isophorone from these applications is expected to be minimal. Isophorone is used as a solvent in the automotive and industrial coatings processes, including food packaging and metal coatings (Felcht U-H. 2006; Montebello 2009).

In Canada, isophorone is permitted as a non-medicinal ingredient for use as a flavour enhancer in licensed natural health products; however, as it is not listed as an ingredient in the licensed natural health products database, it is not found in current licensed natural

health products (2009 personal communication from Natural Health Products Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced).

It is possible that isophorone is used as a flavour in foods sold in Canada. Food flavours are not regulated as food additives, nor are they required under the *Food and Drug Regulations* to undergo a pre-market review (2009 personal communication from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced).

Isophorone use in cosmetics is prohibited in Canada (Health Canada 2007).

As recently as 2009, there was one post-emergent pesticide available for commercial use in Canada that contained isophorone as a formulant. However, the product registration expired on December 31, 2009, with phase-out to be completed by December 31, 2013 (2010 personal communication from Pest Management Regulatory Agency, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced). In the United States, isophorone is permitted in pesticide formulations that have restricted use (US EPA 2006).

Releases to the Environment

In response to a notice issued under section 71 of CEPA 1999 for 2006, 6 537 kg of isophorone was reported to be released to the atmosphere in 2006, whereas 587 kg of the substance was transferred to hazardous waste facilities. Based on section 71 responses, there were no releases to either land or water and no transfers to non-hazardous waste facilities (Environment Canada 2008).

Isophorone was not listed in either the National Pollutant Release Inventory for the 2001–2008 reporting years (NPRI 2009) or the US Toxics Release Inventory Program for the 2001–2006 reporting years (TRI 2009).

Environmental Fate

The results of Level III fugacity modelling, based on information on physical and chemical properties, indicate that isophorone will reside predominantly in the medium to which it is released (Table 3).

Table 3. Results of Level III fugacity modelling (EQC 2003) for isophorone

Substance released (100%) to:	Fraction of substance partitioning to each medium (%)			
	Air	Water	Soil	Sediment
Air	80.30	8.82	10.9	0.02
Water	0.01	99.80	0.001	0.18
Soil	0.02	5.66	94.3	0.01

Persistence and Bioaccumulation Potential

Environmental Persistence

Results from several empirical degradation tests with isophorone are summarized in OECD (2003). In a test for ready biodegradability, 95% degradation was observed after 28 days, indicating that the ultimate degradation half-life of the substance is much less than 182 days (6.5 days, assuming first-order degradation kinetics). Similarly, in a test of inherent biodegradability, 89% of the substance was degraded after 14 days; in a 33-day simulation test, 69% degradation was noted.

Some empirical biodegradation data (NITE 2002) show very low biodegradation of isophorone over 28 days in a ready biodegradation test. However, as stated in OECD (2003), tests on the toxicity of isophorone to microorganisms show inhibitory effects at the initial concentration used for testing (100 mg/L) and therefore, the NITE (2002) ready biodegradability test results are considered to be invalid (OECD 2003).

To complement the experimental data for the degradation of isophorone, a quantitative structure–activity relationship (QSAR)-based weight of evidence approach (Environment Canada 2007) was applied. Table 4 summarizes the results of available QSAR models for degradation in air and water. Isophorone does not contain functional groups expected to undergo hydrolysis.

Table 4. Modelled data for degradation of isophorone

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2000	$t_{½}$ = 0.133 day	<2
Ozone reaction	AOPWIN 2000	$t_{½}$ = 0.155 day	<2
Water			
Hydrolysis	HYDROWIN 2000	n/a[1]	n/a
Biodegradation (aerobic)	BIOWIN 2000 Submodel 3: Expert Survey (ultimate biodegradation)	2.66[2] "weeks to months"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 4: Expert Survey (primary biodegradation)	3.47[2] "days to weeks"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 5: MITI linear probability	0.53[3] "biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 6: MITI non-linear probability	0.58[3] "biodegrades fast"	<182
Biodegradation (aerobic)	TOPKAT 2004 Probability	0.0[3] "biodegrades slowly"	≥182
Biodegradation	CATABOL ©2004–2008	% BOD = 12	≥182

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
(aerobic)	% BOD	"biodegrades slowly"	

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; n/a, not applicable; $t_{½}$, half-life.
[1] Model does not provide an estimate for this type of structure.
[2] Output is a numerical score.
[3] Output is a probability score.

In air, a predicted atmospheric oxidation half-life value of 0.133 day (see Table 4) suggests that isophorone is likely to be rapidly oxidized by reaction with hydroxyl radicals. Furthermore, a predicted ozone reaction half-life of 0.155 day (see Table 4) suggests that this substance is also likely to be rapidly oxidized by ozone. Isophorone is not likely to degrade via direct photolysis. Therefore, it is expected that reactions with hydroxyl radicals and ozone will be the most important fate processes in the atmosphere. With half-lives of 0.133 and 0.155 day via reaction with hydroxyl radicals and ozone, respectively, isophorone is considered to be not persistent in air.

There are conflicting model results for biodegradation. Two of the four ready biodegradation models (CATABOL and TOPKAT) indicate that biodegradation is very slow and that the half-life in water would be ≥182 days, whereas four BIOWIN submodels indicate a half-life of <182 days (Environment Canada 2009).

Because of the conflicting model results, it is difficult to evaluate persistence based on modelled data alone. However, as noted previously, there is clear evidence from empirical biodegradation testing that the biodegradation half-life of isophorone in water is much less than 182 days (6.5 days, assuming first-order degradation kinetics).

Using an extrapolation ratio of 1:1 for water:soil biodegradation half-lives (Boethling et al. 1995) and the half-life of 6.5 days in water estimated from results of the 28-day ready biodegradation test, the ultimate biodegradation half-life in soil is also ≤182 days. Using an extrapolation ratio of 1:4 for water:sediment biodegradation half-lives (Boethling et al. 1995), the half-life in sediment is ≤365 days. These results indicate that isophorone is not expected to be persistent in soil or sediment.

Based on the modelled data, isophorone does not meet the persistence criteria in air, water, soil or sediment (half-life in air ≥2 days, half-lives in soil and water ≥182 days and half-life in sediment ≥365 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

A low experimental log K_{ow} value of 1.67 for isophorone suggests that this chemical has low potential to bioaccumulate in biota (see Table 2).

Table 5a presents two empirical bioconcentration factor (BCF) values for fish. No other experimental bioaccumulation data were identified.

Table 5a. Empirical data for bioaccumulation of isophorone

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Oziras latipes (Rice fish)	BCF	1.1–1.8	NITE 2002
Lepomis macrochirus (Bluegill sunfish)	BCF	7	Veith et al. 1980

As few empirical bioaccumulation data were available for isophorone, a predictive approach was applied using available bioaccumulation factor (BAF) and BCF models, as shown in Table 5b.

Table 5b. Modelled data for bioaccumulation of isophorone

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Fish	BAF	2.05[1]	Arnot and Gobas 2003 (Gobas BAF middle trophic level)
Fish	BCF	1.97	Arnot and Gobas 2003 (Gobas BCF middle trophic level)
Fish	BCF	4.42	BBM 2008
Fish	BCF	6.14	BCFWIN 2000

[1] Corrected for metabolic degradation.

The modified Gobas BAF middle trophic level model for fish (taking into account metabolic degradation) predicted a BAF of 2.05 L/kg, indicating that isophorone does not have the potential to bioaccumulate in fish and to biomagnify in food webs. The results of BCF model calculations provide additional data supporting the low bioaccumulation potential of this substance.

Based on the available empirical and kinetic-based modelled values, isophorone does not meet the bioaccumulation criteria (BAF or BCF ≥5 000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Thee are modelled predictions and experimental evidence that suggest isophorone has the potential to exert only low to moderate toxicity to aquatic organisms following short-term (acute) exposure. Although modelled calculations for aquatic toxicity were performed for this substance, they are not needed for this assessment, given the numerous experimental data available (see Table 6). The modelled values—ranging from 36 mg/L (acute median lethal concentration [LC_{50}] for fish) to 798 mg/L (acute median effective concentration [EC_{50}] to water flea)]—are, to a fair degree, in concurrence with the experimental results.

Table 6. Empirical data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Green alga	Acute (72 h)	EC_{10}	64[1]	Hüls AG 1996a

		EC_{50}	475[1]	
Water flea	Acute (24 h)	EC_0	90	Hüls AG 1996b
		EC_{50}	254	
Water flea	Acute (24 h)	LC_{50}	430	LeBlanc 1980
	Acute (48 h)	LC_{50}	120	
		NOEC	15	
Water flea	Acute (48 h)	LC_{50}	117[2]	LeBlanc 1980
Fathead minnow	Chronic (35 days)	LOEC	19	Cairns and Nebeker 1982
Fathead minnow	Chronic (35 days)	NOEC	11	Cairns and Nebeker 1982
Fathead minnow	Acute (96 h)	LC_{50}	145[1]	Cairns and Nebeker 1982
			255[1]	Cairns and Nebeker 1982
			228[3]	Geiger et al. 1990
Fathead minnow	Acute (96 h)	EC_{50}	217[1]	Geiger et al. 1990
Fathead minnow	Acute (96 h)	LC_{50}	240	Brooke 1991
Fathead minnow	Acute (96 h)	LC_{50}	253	Brooke 1991
Fathead minnow	Acute (96 h)	LC_{50}	275	Brooke 1991
Fathead minnow	Acute (96 h)	LC_{50}	319	Brooke 1991
Japanese medaka	Acute (48 h)	LC_{50}	340[1]	MITI 1992
Golden ide	Acute (48 h)	LC_{50}	209[1]	Hüls AG 1996c
Bluegill	Acute (96 h)	LC_{50}	220	Buccafusco et al. 1981

Abbreviations: EC_x, concentration of a substance that is estimated to cause some toxic sublethal effect on x% of the test organisms; LC_{50}, concentration of a substance that is estimated to be lethal to 50% of the test organisms; LOEC, lowest-observed-effect concentration; NOEC, no-observed-effect concentration.

[1] Nominal concentration.

[2] Pivotal iT value chosen for categorization.

[3] Effective concentration.

A range of acute aquatic toxicity values was obtained from the various experimental studies that were identified. These results indicate that the substance is not highly hazardous to aquatic organisms (acute LC_{50}/EC_{50} >1.0 mg/L).

The effect of isophorone on four crops (cotton, soybean, corn and wheat) was also studied (Krenek and King 1987). Undiluted isophorone was sprayed at a dose of 3.27 mL/m^2. Although some leaves were damaged a few hours after application, all plants showed evidence of recovery after 56 hours.

No recent monitoring data for the concentration of isophorone in Canadian surface water bodies were found, although there are limited historical monitoring data. A concentration of 69 ng/L in the St. Lawrence River in 1987 was reported (Germain and Langlois 1988). No monitoring data for the presence of isophorone in Canadian air or sediment have been

identified. In 1992, "trace" amounts (unquantified) of isophorone were found in 30 agricultural soil samples treated with wastewater treatment plant sludge from Hamilton, Ontario (Webber 1994). Monitoring data from other countries (e.g., United States) have been reported; the highest concentration reported (10 µg/L) was detected in urban runoff in Washington, DC (OECD 2003).

Based on information received under section 71 of CEPA 1999, releases of isophorone to outdoor air are occurring in Canada (Environment Canada 2008). These are point source emissions occurring at industrial facilities where isophorone is used as a solvent. Furthermore, isophorone has a half-life in air of 0.133–0.155 day and is therefore not considered to be persistent in this medium. Consequently, atmospheric emissions of isophorone are not likely to be causing harm.

Given the current uses of isophorone, releases to water could also occur. Environment Canada's Industrial Generic Exposure Tool – Aquatic (Environment Canada 2007) was therefore used to conservatively estimate local exposure in the vicinity of a potential industrial source of release to water. The site-specific scenario is based on the largest amount used at a single industrial facility and assumes that conservative fractions are released to water and removed at the sewage treatment plant and that the subsequent discharge is to a relatively small receiving water body. This exposure tool yielded a predicted environmental concentration (PEC) of 0.15 mg/L. Details regarding the inputs used to estimate this concentration and the output of the model are described in Environment Canada (2008b).

A conservative predicted no-effect concentration (PNEC) was derived from the geometric mean of the lowest empirical toxicity values identified. The critical toxicity values for this assessment are the 35-day no-observed-effect concentration (NOEC) (11 mg/L) and lowest-observed-effect concentration (LOEC) (19 mg/L) for chronic toxicity to the fathead minnow (Cairns and Nebeker 1982). The geometric mean is 14.5 mg/L. An application factor of 10 is used to account for uncertainty in extrapolating from laboratory to field conditions and for intraspecies and interspecies variations in sensitivity, giving a PNEC of 1.45 mg/L.

The resulting conservative risk quotient (PEC/PNEC) of 0.1 for an industrial scenario indicates that these exposure values are unlikely to cause harm to aquatic organisms.

The approach taken in this ecological screening assessment was to examine various scientific and technical information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substance.

Isophorone is not expected to be persistent in air, water, soil or sediment, and it is also expected to have a low bioaccumulation potential. The high importation volumes of isophorone into Canada, along with information on its uses, indicate potential for widespread release into the Canadian environment. Once released into the environment,

isophorone will be found mainly in air and water. It has also been demonstrated to have low to moderate potential for toxicity to aquatic organisms. A risk quotient analysis integrating conservative estimates of exposure with toxicity information was performed for the aquatic medium, resulting in a risk quotient (PEC/PNEC) of 0.1. Therefore, harm to aquatic organisms is unlikely.

This information indicates that isophorone is unlikely to cause ecological harm in Canada.

It should be noted that this conclusion was reached despite the conservative assumptions that were made in response to uncertainties encountered in the assessment. One uncertainty relates to the lack of empirical data for environmental concentrations in Canada, which was addressed by predicting an upper-bounding concentration in water using an exposure model. Regarding ecotoxicity, the significance of soil as a potentially important medium of exposure is not well addressed by the effects data available, but exposure in this medium is not expected to be significant.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media and Food

Upper-bounding estimates of isophorone intake from environmental media and food for each age group in the general population of Canada are presented in Appendix 1a. These upper-bounding estimates of intake range from 0.03 µg/kg body weight (kg-bw) per day (0–6 months old [formula fed]) to 0.84 µg/kg-bw per day (20–59 years old). For the majority of the age groups, food and beverages were estimated to be the major contributors to isophorone exposure, albeit at very low levels, based on non-Canadian data for isophorone in foods (including beverages).

A survey of isophorone levels in Canadian ambient air was not identified in a review of the literature. However, isophorone was screened for but not detected (detection limit not stated) in a US survey of ambient air levels of 189 hazardous air pollutants conducted from 1967 through 1992 (Kelly et al. 1994). In a 1984 study of a contaminated industrial site, isophorone was present in the wastewater but not in the headspace above the wastewater or in ambient air samples taken at nearby urban and rural locations (Hawthorne and Sleevers 1984). The short half-life of 0.133 day may account for the absence of atmospheric monitoring data (ATSDR 1989).

In the absence of experimental data, an isophorone concentration in outdoor air of 7.63×10^{-5} µg/m^3 was estimated based on an atmospheric release of 6 537 kg of isophorone in 2006 (Environment Canada 2008) and using exposure modelling software (ChemCAN 2003). This modelled estimate was used to determine the upper-bounding exposure estimate from ambient air.

One Canadian study reported isophorone levels in indoor air (Otson and Benoit 1986). In this study, 10 homes in Montreal, Quebec, were sampled in the fall and winter of 1983–1984. Based on occupant habits, houses were designated as either smoking or non-smoking. Isophorone was not detected in samples taken from any of the homes. The detection limit of 5 ng/m^3 from this study was used as a conservative value for calculating the upper-bounding estimate of exposure to isophorone in indoor air. No other Canadian or US studies were located.

No reports of detectable amounts of isophorone were found in drinking water surveys from Toronto, Ontario, for the periods January 2001 through September 2003 (City of Toronto, 2001, 2002, 2003). Isophorone levels reported in drinking water from New Orleans, Louisiana, in 1978 ranged from 1.5 to 2.9 μg/L (US EPA 1978).

The detection limit of 0.3 μg/L from the Toronto reports was used to calculate upper-bounding estimates of exposure to isophorone in drinking water, as the data were from a recent Canadian study (City of Toronto 2001, 2002, 2003).

In 1992, 30 agricultural soil samples treated with wastewater treatment plant sludge from Hamilton, Ontario, were analysed for industrial organic compounds, including isophorone. One sample was reported to contain a "trace" amount, but the amount was not quantified (detection limit 0.09 mg/kg dry weight) (Webber 1994).

In the absence of more recent soil data, the detection limit of 0.09 mg/kg dry weight from the Webber (1994) study was used for estimating the upper-bounding daily intake of isophorone from soil.

Studies of municipal sludge and sludge composts across Canada from 1993 to 1996 reported no incidence of isophorone. One hundred and two samples were analysed, with the maximum detection limit being 7.0 mg/kg dry weight (Webber and Nichols 1995; Webber and Bedford 1996). More recent Canadian data were not identified.

In the United States, isophorone was quantified at 489 μg/kg in the electrostatic precipitator fly ash from a coal-burning power station. In contrast, isophorone was not detected (detection limit not stated) in the wet scrubber fly ash generated from the same facility (Harrison et al. 1985).

No studies reporting isophorone in Canadian foods were identified. However, isophorone was reported in various food items from food in Japan (Kataoka et al. 2007). The authors noted that isophorone was present in some packaged foods at levels ranging from 2×10^{-5} to 13×10^{-3} μg/g, but not in fresh vegetables (detection limit 5×10^{-7} μg/g).

In the United States, eight species of fish were collected from Lake Michigan estuaries that were known to be contaminated by pollutants from commercial sources. The samples, collected in 1983, had a mean isophorone concentration of 1.17 mg/kg wet

weight. The maximum isophorone concentration was 3.61 mg/kg wet weight, based on 14 composite fish samples from 140 fishes (Camanzo et al. 1987).

In Europe, isophorone has been reported in saffron at levels as high as 254 µg/g dry weight and in honey at levels ranging from 0.05 to 1.45 µg/g (mean concentration 0.36 µg/g) (Guyot et al. 1999; Lechtenberg et al. 2008).

In the absence of current Canadian data, information on the occurrence of isophorone in food from the above non-Canadian studies (Camanzo et al. 1987; Guyot et al. 1999; Kataoka et al. 2007; Lechtenberg et al. 2008) was included in the calculations for estimating upper-bounding exposure from food. For most food groups, the isophorone intake from the group was estimated using the highest concentration of isophorone that was identified from the foods that could reasonably be assigned to the group. For food categories in which isophorone was not detected in a food, the analytical detection limit was used as the concentration of isophorone in the food category. Food items in which isophorone concentrations were measured are tabulated in Appendix 1b.

Based on information received from section 71 surveys, isophorone may be present in food packaging (Environment Canada 2008). Specifically, isophorone may be used as a processing aid in the manufacture of some linings or coatings for beverage cans, metal spice tins or other food packaging (Montebello 2009; 2009 personal communication from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced). Isophorone is not commonly used as a solvent for food can coatings. If it were to be used, it would not be expected to remain in the properly dried and cured coating. However, considering a conservative scenario where a minimum residual level of isophorone would remain in the coating, a probable daily intake of isophorone from beverage cans was estimated to be $\leq 3.6 \times 10^{-3}$ µg/kg-bw per day (2009 personal communication from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced). The contribution of this source to total intake was considered to be negligible. A probable daily intake estimate for the spice tins application was considered not necessary due to the fact that significant migration to dry foods is not expected (2009 personal communication from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced).

The extent to which the exposure assessment in this report captures the presence of isophorone as a result of its use as a flavour in foods sold in Canada, if such use exists, is not known. Most of the data on the levels of isophorone in foods were taken from the Japanese study (Kataoka et al. 2007), and the source of the isophorone in the foods in that study was not conclusively identified in the paper that reported the results.

For most flavours, including isophorone, there are no provisions in the *Food and Drug Regulations* that control their addition to foods, although such use must not result in a violation of a food's standard of identity and composition in the Regulations or of section 4 of the *Food and Drugs Act*. "Usual" uses for isophorone as a food flavour ranging from 0.50 to 16.80 parts per million (ppm) have been reported (FEMA 1994), but it is unknown if such uses reflect the Canadian situation (2009 personal communication from

Food Directorate, Health Canada, to Risk Assessment Bureau, Health Canada, unreferenced).

Isophorone is used primarily as an industrial substance (ATSDR 1989; Kelly et al. 1994). According to information obtained from submissions under section 71 of CEPA 1999, isophorone is released only to the atmosphere in Canada (Environment Canada 2008). The short atmospheric half-life (<5 hours) of isophorone and Canadian drinking water data suggest that environmental exposure of the general population in Canada to the substance is minimal (ATSDR 1989; City of Toronto 2001, 2002, 2003).

Confidence in the assessment of environmental exposure is moderate, based on a limited number of recent Canadian studies that are available. Exposure to isophorone from ambient air was estimated using exposure modelling software, but confidence is high in the low estimate obtained, because isophorone was not detected in the headspace above a contaminated wastewater site in the United States (Hawthorne and Sleevers 1984). In addition, isophorone is believed to have a short atmospheric half-life and therefore, would not be expected to persist in air.

Consumer Products

According to section 71 submissions, the main isophorone activity in Canada is for the processing of industrial paint and coating formulations (Environment Canada 2008). Isophorone is not part of the formulation for consumer paints or coatings (2009 personal communication from Environment Canada to Risk Assessment Bureau, Health Canada). Once dried and cured, any residual isophorone remaining in the hardened surface is at an extremely low concentration, which is not considered to be hazardous to the consumer.

In Canada, isophorone is not permitted in cosmetic products (Health Canada 2007). However, in Denmark, where isophorone use in cosmetic products was reported, isophorone levels were not a concern for health (Svendsen et al. 2005).

The presence of isophorone in consumer products is not expected to be at a significant level. In Canada, isophorone use is limited, and exposure beyond industrial settings is expected to be at very low residual levels.

Health Effects Assessment

An overview of health effects information for isophorone is presented in Appendix 2.

The European Commission has classified isophorone as a Category 3 carcinogen (*causes concern for humans owing to possible carcinogenic effects*) with risk phrase 40 (*limited evidence of a carcinogenic effect*), based on limited evidence of carcinogenicity in animal studies (European Commission 1998a, b; ESIS 2009). The US Environmental Protection Agency (US EPA) classifies isophorone as a Category C carcinogen (*possible human carcinogen*), based on no data in humans and limited evidence of carcinogenicity in laboratory animals (US EPA 1992).

The carcinogenicity of isophorone was investigated in a 2-year oral (gavage) study in both sexes of rats and mice, at doses of 0, 250 and 500 mg/kg-bw per day. There was evidence of carcinogenicity of isophorone in male Fischer 344 rats, as shown by a statistically significant increase of renal tubular cell adenomas and adenocarcinomas (combined) at 250 and 500 mg/kg-bw per day and carcinomas of the preputial gland at the high dose. In male B6C3F1 mice, there was a statistically significant increase in the incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumours (fibroma, sarcoma, fibrosarcoma and neurofibrosarcoma combined) of the integumentary system at 500 mg/kg-bw per day. An increased incidence of lymphomas or leukemias was noted in low-dose male mice only. No evidence of carcinogenicity was seen in female rats or female mice (NTP 1986). No inhalation carcinogenicity bioassays or human epidemiological studies were identified.

Isophorone was not genotoxic in *in vivo* studies or in the majority of *in vitro* assays in mammalian cells and bacteria. *In vivo* assays on chromosomal damage, deoxyribonucleic acid (DNA) binding in liver and kidney and sex-linked recessive lethal mutations were all negative (Hossack et al. 1978a; Microbiological Associates 1984a; O'Donoghue et al. 1988; Thier et al. 1990; Foureman et al. 1994). *In vitro* assays for mutagenicity, chromosomal aberrations and DNA damage or repair in mammalian cells were primarily negative (Microbiological Associates 1984b, c; NTP 1986; O'Donoghue et al. 1988; Gulati et al. 1989; Honma et al. 1999a). Ames tests for mutagenicity in *Salmonella typhimurium* with and without metabolic activation were negative (Hossack et al. 1978b; Mortelmans et al. 1986; NTP 1986; Hüls 1988a). There were several positive results in mammalian cell assays *in vitro*, but only at cytotoxic concentrations or in non-standard studies (NTP 1986; Tennant et al. 1987; McGregor et al. 1988; Gulati et al. 1989; Ono et al. 1991; Selden et al. 1994; Matsuoka et al. 1996; Honma et al. 1999a, b; Yasunaga et al. 2004). The weight of evidence of all genotoxicity data, especially the negative *in vivo* results, indicates that isophorone is not genotoxic and suggests that it is not a DNA reactive compound. Other national and international agencies have concluded that isophorone is not mutagenic (IPCS 1995a; OECD 2003; US EPA 2006).

The mode of action for the isophorone-induced kidney tumours in male rats has been associated with α2u-globulin (Strasser et al. 1988; ATSDR 1989; Short 1993; Tennant 1993; IPCS 1995a; OECD 2003; Lock and Hard 2004; US EPA 2008). However, α2u-globulin is not detected at significant levels in plasma and urine of female rats, male or female mice, or humans (Swenberg et al. 1989). Chemicals that induce α2u-globulin nephropathy in male rats bind to the protein in the liver; these conjugates are difficult to hydrolyse and induce the formation of hyaline droplets, which accumulate in the tubules, resulting in a nephrotoxic response and a sustained increase in cell turnover (Swenberg et al. 1989, 1992). Isophorone and its proposed metabolites isophorol and dihydroisophorone bind to α2u-globulin *in vivo*, resulting in an increased accumulation of hyaline droplets in renal tubular cells (Strasser et al. 1988; Saito et al. 1996). Administration of isophorone has induced renal tubular tumours in male rats, but not in female rats or either sex of mice (NTP 1986). Detailed review and analysis by the US EPA showed that the isophorone-induced male rat kidney tumours met their criteria for

associating renal tumours with α2u-globulin (US EPA 1991a, b, 2008). Therefore, the increase in the incidence of renal tubular tumours observed in male rats following isophorone administration was caused by the accumulation of α2u-globulin and was sex and species specific; hence, it is likely not relevant to humans (Strasser et al. 1988; ATSDR 1989; Tennant 1993; IPCS 1995a; OECD 2003; US EPA 2008).

The significance of the apparent higher incidence of preputial gland tumours that was observed only in male rats in the high dose group (500 mg/kg-bw per day) cannot be determined. As the gland was investigated histopathologically only when gross lesions were found, the actual incidence of all types of proliferative lesions of the prepuce in either the historical or concurrent controls is not precisely known (NTP 1986). As high levels of α2u-globulin are present in the preputial gland of both male and female rats (Murthy et al. 1987), the isophorone-induced preputial gland tumours in male rats may also have been associated with α2u-globulin accumulation (IPCS 1995a, b).

In terms of liver tumours, only high-dose male mice showed an increased incidence of combined adenomas and carcinomas. Female mice and rats of both sexes did not show any evidence of increased incidence of liver tumours (NTP 1986). Increased incidences of mesenchymal tumours of the integumentary system were shown in high-dose male mice only; the marginally increased incidence of lymphomas or leukemias was observed only in low-dose male mice, without dose–response evidence. The high mortality in male mice also makes the results difficult to evaluate. Although the liver and mesenchymal tumours may be treatment related, there is no clear evidence that the lymphomas or leukemias in male mice were treatment related. Also, there is evidence to indicate that isophorone does not bind to DNA *in vivo* (Thier et al. 1990), suggesting that isophorone may exert its toxic effects through a mechanism other than direct interaction with DNA (Topping et al. 2001). In addition, the US EPA considered the carcinogenicity test results in male mice to be "equivocal" (US EPA 2008).

The lowest inhalation LOEC in laboratory animals has been identified as 208 mg/m^3 (36 ppm) from a 4-week study in male and female rats, based on reduced growth and liver weights in male rats and blood effects (increased lymphocytes and decreased neutrophils) in both sexes of rats (Exxon 1968). In a subchronic inhalation study, increased mortality was observed in rats exposed to isophorone at 2873 mg/m^3 (500 ppm) for 4 or 6 months (only one exposure level tested) (Dutertre-Catella 1976). Microvacuolization of the liver in rats and rabbits was observed in a chronic inhalation study, following 18 months of exposure to isophorone at 1436 mg/m^3 (250 ppm; only one exposure level tested) (Dutertre-Catella 1976).

In humans, complaints of fatigue and malaise from employees exposed for 1 month to isophorone concentrations ranging from 28 to 45 mg/m^3 (5 to 8 ppm) were reported; the complaints stopped when isophorone concentrations decreased to 6–22 mg/m^3 (l–4 ppm) (Ware 1973). However, no further details were available, including exposure conditions of the employees and potential co-exposures.

By the oral route, the lowest-observed-effect level (LOEL) for chronic exposure is 250 mg/kg-bw per day, based on a statistically significant increase in the incidence of female rats with nephropathy and increased incidences of coagulative liver necrosis and hepatocytomegaly in male mice in the 2-year study (NTP 1986). At a dose level of 1000 mg/kg-bw per day, reduced body weight gain in male rats and increased mortality in female mice were observed in a subchronic (90 days) study (NTP 1986). In another subchronic study, no toxicologically relevant effects were observed at doses up to 150 mg/kg-bw per day (except for a mild intermittent incidence of soft stools at 75 and 150 mg/kg-bw per day), which was the highest dose administered to Beagle dogs for 90 days (Rohm & Haas Co. 1972). For 16-day exposures, the lowest LOEL was 250 mg/kg-bw per day, based on reduced body weight gain in female mice. In the same study, at 1000 mg/kg-bw per day, sluggishness and lethargy in rats and staggering in mice were observed (NTP 1986).

In terms of dermal exposure, the lowest LOEL of 658 mg/kg-bw per day was identified based on erythema and crusted skin in the only repeated-dose dermal exposure study identified, in which isophorone was applied daily for 8 weeks to shaved and abraded (occluded) skin of rats (Dutertre-Catella 1976).

Developmental effects following isophorone exposure were observed only above the concentrations associated with maternal toxicity. There was evidence of maternal toxicity at 664 mg/m^3 (115 ppm), but there were no developmental effects observed at this exposure level (Exxon 1984a). Teratogenicity (exencephaly) was observed at 866 mg/m^3 (150 ppm) in a few mouse and rat fetuses in the pilot study (Exxon 1984b). However, this dose exceeded the concentration that was shown to cause maternal toxicity in the main study (Exxon 1984a). Therefore, isophorone does not appear to be a developmental toxicant.

Isophorone did not influence litter sizes, nor were there any abnormalities observed in the pups in a limited one-generation study in Wistar rats exposed by inhalation to isophorone at 2873 mg/m^3 (500 ppm) (Dutertre-Catella 1976). However, only one concentration was used, the group size was small and no information was provided on reproductive success. No other studies on reproductive toxicity were identified. In 90-day oral studies, no changes were observed in histopathological examinations of the reproductive organs in Beagle dogs exposed to up to 150 mg/kg-bw per day (Rohm & Haas Co. 1972) or in rats exposed to up to 1000 mg/kg-bw per day (NTP 1986). Overall, isophorone does not appear to be a reproductive toxicant.

Irritation effects of isophorone were observed in both humans and experimental animals. In two investigations with 6 and 12 volunteers, throat irritation was reported at greater than 199 mg/m^3 (Esso 1965a), and eye and nasal irritation at concentrations greater than 230 mg/m^3, following acute exposure (Smyth and Seaton 1940). In a further investigation with 12 volunteers exposed to isophorone vapours for 15 minutes, eye, nose and throat irritation was reported at 144 mg/m^3 (Silverman et al. 1946). The results from animal bioassays on the irritating effects of isophorone have been consistent with observations in humans. Isophorone was found to be irritating to eyes at 0.1 mL undiluted (40 mg/kg-bw)

and irritating to skin at 0.5 mL undiluted (200 mg/kg-bw) in rabbits (Esso 1964; Truhaut et al. 1972; Dutertre-Catella 1976).

Internationally, isophorone has been classified as an irritant to eyes and the respiratory system by the European Commission (2008).

Limited toxicokinetic studies suggest that after oral and inhalative administration, isophorone is well absorbed and rapidly distributed in rats and rabbits (Dutertre-Catella 1976). Systemic effects in acute toxicity studies indicate absorption following dermal exposure (Günzel and Richter 1968b). Methyl-oxidation, glucuronidation and hydrogenation appear to be the major transformation processes for isophorone (Truhaut et al. 1970; Dutertre-Catella et al. 1978). The tendency of isophorone to bioaccumulate is very low, as 80% of orally administered isophorone was excreted by rats within 96 hours (Thier 1991).

Confidence in the toxicity dataset is considered to be moderate to high. Data are available for acute toxicity, repeated-dose toxicity, chronic toxicity and carcinogenicity, and genetic toxicity. However, limited data are available on long-term inhalation, reproductive and developmental toxicity. There is only one chronic oral study using only two doses other than control. The only subchronic or chronic inhalation study used only one very high isophorone concentration and had small group sizes.

Characterization of Risk to Human Health

Carcinogenicity was considered in the health effects assessment for isophorone, as the substance had been classified as carcinogenic by some national or international agencies (i.e., European Commission and US EPA), but not others. These classifications were based upon the results of two animal bioassays of sufficient quality, as no sufficient epidemiological studies were available. Apparently increased incidences of tumours at multiple sites (kidney, preputial gland, liver and skin) were observed in 2-year standard carcinogenicity studies with rats and mice (NTP 1986). The induction of kidney tumours following isophorone administration was caused via the accumulation of α2u-globulin, a mechanism not relevant to humans (US EPA 1991a; IPCS 1995a; OECD 2003; US EPA 2008). Consideration of the available information regarding genotoxicity and the conclusions of other agencies indicate that isophorone is not likely to be genotoxic. Accordingly, although the mode of induction of tumours is not fully elucidated, the tumours observed in rodents are not considered to have resulted from direct interaction with genetic material. Therefore, a threshold approach is used to assess risk to human health.

For oral exposures, the lowest reported LOEL was 250 mg/kg-bw per day, based on non-neoplastic effects observed after chronic exposure (nephropathy in female rats; liver necrosis, hepatocytomegaly in male mice) in a 2-year toxicology and carcinogenicity study, whereas the potentially chemical-related increase in tumour incidence in that study was observed only at higher doses (NTP 1986).

Comparison of the critical effect level for repeated dosing via the oral route (i.e., the LOEL of 250 mg/kg-bw per day) with the upper-bounding estimate of daily intake of isophorone by the most highly exposed group (20–59 years) via environmental media and food in Canada (0.84 μg/kg-bw per day) results in a margin of exposure of approximately 298 000. Taking into account reasonable, upper-bounding estimates of exposure, the margin of exposure is considered adequately protective of human health for both cancer and non-cancer effects.

Oral ingestion, from food and drinking water, was identified as the predominant route of exposure for isophorone to the general population of Canada. Intake estimates from other sources were not as significant (Appendix 1a).

Uncertainties in Evaluation of Risk to Human Health

This screening assessment does not take into account potential human variability, nor does it consider differences between humans and experimental animals in terms of sensitivity to the potential effects of isophorone. There is not enough information to evaluate the incidences of preputial gland tumours in rats, because the true tumour incidence is not available in either historical data or the identified study (NTP 1986). Preputial gland tumours were not seen in mice, suggesting that this type of tumour may be species specific, and the relevance of rat preputial gland tumours to humans is unknown. The available study showed equivocal evidence of liver tumours in mice. The evidence for carcinogenicity is limited, but the consistently strong negative database of *in vivo* genotoxicity studies suggests that isophorone is not mutagenic.

Lack of recent Canadian data regarding levels of isophorone in environmental media and food is a source of uncertainty in the upper-bounding multimedia exposure estimates for the general population of Canada. However, concern about this uncertainty is reduced by taking into account the large margin of exposure between the critical effect level and the highest estimated intake of isophorone. Confidence is high that the derived multimedia exposure estimates are adequately protective of the general population of Canada, as conservative assumptions were used when recent Canadian data were unavailable.

Conclusion

Based on the information presented in this screening assessment, it is concluded that isophorone 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.

Based on comparison of the upper-bounding estimated exposure to isophorone in Canada with the critical effect level, it is considered that the resulting margin of exposure is adequately protective of human health. It is concluded that isophorone is not 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 isophorone does not meet any of the criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

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Appendix 1a: Upper-bounding estimates of daily intake of isophorone by the general population in Canada

Route of exposure	Estimated intake (µg/kg-bw per day) of isophorone by various age groups							
	0–6 months[1]			**0.5–4 years[4]**	**5–11 years[5]**	**12–19 years[6]**	**20–59 years[7]**	**60+ years[8]**
	Breast fed[2]	**Formula fed[3]**	**Not formula fed**					
Ambient air[9]	$<1\times10^{-5}$			$<1\times10^{-5}$	$<1\times10^{-5}$	$<1\times10^{-5}$	$<1\times10^{-5}$	$<1\times10^{-5}$
Indoor air[10]	0.001			0.003	0.002	0.001	0.001	0.001
Drinking water[11]	N/A	0.03	0.01	0.01	0.01	0.01	0.01	0.01
Food and beverages[12]	N/A	<0.003	0.13	0.53	0.31	0.20	0.83	0.20
Soil[13]	$<1\times10^{-4}$			$<1\times10^{-4}$	$<1\times10^{-4}$	$<1\times10^{-4}$	$<1\times10^{-4}$	$<1\times10^{-4}$
Total intake	N/A	0.03	0.14	0.55	0.32	0.21	0.84	0.20

Abbreviation: N/A, not applicable.

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).

[2] No data on detectable levels of isophorone in breast milk were located.

[3] For exclusively formula-fed infants, intake from water is that amount required to reconstitute formula. No data on isophorone levels in formula were found; however, concentrations of isophorone in drinking water were used in this model (see footnote 11). Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW 1990).

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

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

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

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

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

[9] Isophorone has been monitored for but not detected in ambient air in the United States. In the absence of experimental data from Canada, a modelled estimate of an isophorone concentration of 7.63×10^{-5} µg/m^3 was used to calculate the upper-bounding estimates for ambient air exposure (ChemCAN 2003). Canadians are assumed to spend 3 h per day outside (Health Canada 1998).

[10] Isophorone has been monitored for in homes in Canada. It was not detected in a study of 10 homes in the Montreal, Quebec, area sampled in triplicate for 20 consecutive days during the fall and winter of 1983–1984. The detection limit of 5×10^{-3} µg/m^3 (Otson and Benoit 1986) was used to calculate maximal daily exposure from indoor air. Canadians are assumed to spend 21 h indoors each day (Health Canada 1998).

[11] No reports of detectable amounts of iisophorone in drinking water sources in Canadian communities were found. The city of Toronto reported a detection limit of 0.3 µg/L and no detectable levels of isophorone from 51 treated samples collected between January 2001 and September 2003 (City of Toronto 2001, 2002, 2003). The detection limit of 0.3 µg/L was used in calculations to estimate maximal exposure from drinking water.

[12] No data were identified for the concentration of isophorone in foods in Canada. Studies from Japan, the United States and Europe had reported levels of isophorone in food available in their respective locations. In the absence of Canadian data, foreign data (Camanzo et al. 1987; Guyot et al. 1999; Kataoka et al. 2007; Lechtenberg et al. 2008)described in Appendix 1b were included in the

calculations for estimating upper-bounding exposure from food. Amounts of foods consumed on a daily basis by each age group are described by Health Canada (1998).

[13] One of 30 samples of soil in Canada was reported to contain a trace concentration of isophorone. The amount was not quantified, so the detection limit from that study, 90 µg/kg dry weight, was used for the calculation of intake of isophorone from soil (Webber 1994).

Appendix 1b: Concentrations of isophorone reported in various food items

Description	Country of origin	Maximum concentration[1,2] (ng/g)
Whole milk	Japan	0.20
Beef, steak	Japan	ND
Pork, fresh	Japan	0.22
Poultry	Japan	0.184
Scallops	Japan	0.238
Eggs, medium	Japan	ND
Fish, fresh	United States	3.61×10^3
Soy sauce	Japan	3.304
Flour, wheat	Japan	1.674
Rice, dry	Japan	2.868
Potatoes	Japan	ND
Peppers	Japan	ND
Carrots, fresh	Japan	ND
Tomato juice, canned	Japan	0.994
Sugar	Japan	1.568
Syrup, pancake	Japan	1.252
Honey	Europe[3]	1.45
Tea	Japan	0.647
Alcohol drink spirits	Japan	0.361
Miscellaneous food (saffron)	Europe[3]	2.54×10^5

Abbreviation: ND, not detected.

[1] Sources: Japan, Kataoka et al. 2007; United States, Camanzo et al. 1987; honey – Europe, Guyot et al. 1999; saffron – Europe, Lechtenberg et al. 2008.

[2] Detection limit for isophorone in Japanese foods was 0.5 pg/g.

[3] Specific European country of origin not identifiable from the reference.

Appendix 2: Summary of health effects information for isophorone

Endpoint	Lowest effect levels[1]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**Lowest inhalation LC_{50} (6 h)** = 3500 mg/m^3 (619 ppm) in female Wistar rats and female Swiss mice (Esso 1964). **Other inhalation LC_{50} (4 h)** = 7000 mg/m^3 (1238 ppm) (confidence interval = 5700–8600 mg/m^3) in rats (Esso 1965b). [Additional acute inhalation studies: Smyth and Seaton 1940; Esso 1964; Dutertre-Catella 1976; Topping et al. 2001] **Lowest oral LD_{50}** = 1500 mg/kg-bw in male and female rats, 5 per sex per group, at doses of 500–2500 mg/kg-bw (Günzel and Richter 1968a). [Additional acute oral studies: Esso 1964; Dutertre-Catella 1976] **Lowest dermal LD_{50}** = 1200 mg/kg-bw in rabbits. Accelerated breathing, prostration, narcosis and erythema were observed at 1200 mg/kg-bw (Dutertre-Catella 1976). [Additional acute dermal studies: Esso 1964; Günzel and Richter 1968b]
Short-term repeated-dose toxicity	**Lowest inhalation LOEC** = 208 mg/m^3 (36 ppm), based on reduced growth and liver weights in male rats and blood effects (increased lymphocytes and decreased neutrophils) in both sexes of rats; increased percentage of lymphocytes in both sexes of mice exposed to 0 or 208 mg/m^3, 6 h/day, 5 days/week, for 4 weeks, but histological examination of the livers of 30% of the rats revealed no treatment-related liver lesions (Exxon 1968). No pathological changes or histological abnormalities were observed in the upper respiratory tract of (Swiss OF1 (IFFA Credo) mice exposed to isophorone at 0 or 164 mg/m^3 (27.8 ppm) (Zissu 1995). [Additional inhalation studies: Smyth et al. 1942] **Lowest oral LOEL** = 250 mg/kg-bw per day, based on reduced body weight gain in females in a study in B6C3F1 mice, 5 per sex per dose, exposed to 0, 125, 250, 500, 1000 or 2000 mg/kg-bw per day in corn oil, 5 days/week for 16 days. At 1000 mg/kg-bw per day, serious neurological effects (rats: sluggish and lethargic; mice: staggering) were observed (NTP 1986). [No additional oral studies identified] **Lowest dermal LOEL** = 658 mg/kg-bw per day in rats, based on formation of erythema and crust on skin after 5–6 weeks of treatment, completely reversed after end of treatment, in a study in groups of male and female Wistar rats exposed to 0.1 mL (658 mg/kg-bw per day) or 0.2 mL (1316 mg/kg-bw per day) undiluted daily on shaved skin for 8 weeks (insufficient information on exposure protocol to identify the dose level at which the effects were observed) (Dutertre-Catella 1976). [No additional dermal studies identified]
Subchronic toxicity	**Lowest inhalation LOEC** = 2873 mg/m^3 (500 ppm), based on increased mortality (1/10 females and 2/10 males) and irritation of eyes and nose in Wistar rats, 10 per sex per group, exposed to 0 or 2873 mg/m^3 6 h/day, 5 days/week, for 4 (females) or 6 months (males) (Dutertre-Catella 1976).

Endpoint	Lowest effect levels[1]/Results
	[No additional inhalation studies identified] **Lowest oral LOEL** = 1000 mg/kg-bw per day, based on slightly reduced body weight gain in male rats and increased mortality in female mice in a study in Fischer 344 rats and B6C3F1 mice, 10 per sex per group, exposed to 0, 62.5, 125, 250, 500 or 1000 mg/kg-bw per day by gavage in corn oil, 5 days/week for 13 weeks. There were no treatment-related effects upon microscopic examination of male and female reproductive organs in both rats and mice (NTP 1986). **Oral NOEL** = 150 mg/kg-bw per day; no effects were observed at up to the highest dose, in Beagle dogs, 4 per sex per dose, administered 0, 35, 75 or 150 mg/kg-bw per day via capsule, 7 days/week for 90 days. Microscopic examination of male and female reproductive organs (testis, seminal vesicle, prostate, ovary, uterus and mammary gland) did not reveal any treatment-related effects (Rohm & Haas Co. 1972 [No additional oral studies identified] No dermal studies identified.
Chronic toxicity/ carcinogenicity	**Oral carcinogenicity in rats:** Groups of male and female rats (50 per sex per dose), Fischer 344, were exposed orally (gavage) to 0, 250 or 500 mg/kg-bw per day (corn oil), 5 days/week for 103 weeks. Male rats showed increased incidences of renal tubular cell adenomas and adenocarcinomas (0/50, 3/50, 3/50), p = 0.12 and 0.025 for low and high doses, respectively, and of preputial gland carcinomas (0/50, 0/50, 5/50), p = 0.012; no evidence of carcinogenicity was seen in female rats (NTP 1986). **Oral carcinogenicity in mice:** Groups of male and female mice (50 per sex per dose), B6C3F1, were exposed orally (gavage) to 0, 250 or 500 mg/kg-bw per day (corn oil) 5 days/week for 103 weeks. The incidences of benign and malignant liver tumors (combined) (18/48, 18/50, 29/50) and mesenchymal tumours of the integumentary system (6/48, 8/50, 14/50) were increased in high-dose males, p = 0.036 and 0.05, respectively. Lymphomas or leukemias were also increased in low-dose males (8/48, 18/50, 5/50), p = 0.046 for lymphoma, p = 0.081 for lymphoma or leukemia (combined), and showed the same increase at the low dose when incidences were adjusted for mortality during the study (adjusted rates were 38%, 63% and 19%, respectively). No evidence of carcinogenicity was seen in females (NTP 1986). **Lowest oral non-neoplastic LOEL** = 250 mg/kg-bw per day, based on a statistically significant increase in incidence of female rats with nephropathy (21/50, 39/50, 32/50 in control, low-dose and high-dose female rats); treated male mice showed increased incidences of coagulative liver necrosis (3/48, 10/50, 11/50 in control, low-dose and high-dose males, respectively) and of hepatocytomegaly (23/48, 39/50, 37/50 in control, low-dose and high-dose males, respectively) (NTP 1986). **Lowest inhalation LOEC** = 1436 mg/m^3 (250 ppm), based on slight irritation of the eyes and nose and microvacuolization of the liver in Wistar rats and New Zealand White rabbits, 10 per sex per group, exposed (further details on exposure regime such as whole body or nose-only were not available) to 0 or 1436 mg/m^3 6 h/day, 5 days/week, for 18 months (Dutertre-Catella 1976). No dermal chronic toxicity/carcinogenicity study identified.
Developmental toxicity	No significant differences among control and treated groups were found for any uterine implantation parameters that were evaluated; for any of the fetal external, visceral or skeletal parameters; or for crown–rump distance and mean body weight in a study in Fischer 344 rats and CD-1 mice, 22 pregnant females per dose, exposed to 0, 144, 289

Endpoint	Lowest effect levels[1]/Results
	or 664 mg/m^3 6 h/day on gestation days 6–15. In the dams of both rats and mice, maternal toxicity, such as significantly reduced food consumption, reduced body weights, alopecia and discoloration of the cervical or anogenital region, was observed at 664 mg/m^3 (115 ppm) (Exxon 1984a). In the pilot study, exencephaly was observed at 866 mg/m^3 (150 ppm) in a few CD-1 mouse and Fischer 344 rat fetuses (further details, such as number of pregnant females, number of fetuses per dose and exposure regime, not available) (Exxon 1984b). [Additional inhalation studies: Dutertre-Catella 1976] No oral or dermal developmental studies identified.
Reproductive toxicity	At 2873 mg/m^3 (500 ppm), reduced parental survival in a one-generation study in which groups of 10 Wistar rats per sex were exposed (further details on exposure regime, such as whole-body or nose-only exposure, not available) to 0 or 2873 mg/m^3 (500 ppm) isophorone, 6 h/day, 5 days/week, for 3 months for males and throughout gestation for females. There were no treatment-related effects on litter size or pup abnormalities, but no information was provided on reproductive success (Dutertre-Catella 1976). No oral or dermal reproduction studies identified.
Genotoxicity and related endpoints: *in vivo*	**Chromosome damage (micronuclei) in bone marrow** **Negative:** in male and female CFLP mice (5 per sex per dose) orally (gavage) administered 0, 225, 450 or 900 mg/kg-bw per day for 2 days; vehicle not specified; bone marrow examined 6 h after second dose (Hossack et al. 1978a) **Negative:** in male and female CD-1 mice (5 per sex per time point) administered intraperitoneally 0 or 500 mg/kg-bw in corn oil; bone marrow examined 12, 24 or 48 h after dosing (Microbiological Associates 1984a; O'Donoghue et al. 1988) **DNA binding in liver and kidney** **Negative:** in male and female B6C3F1 mice (25 per sex per group) and F344 rats (5 per sex per group) orally (gavage) administered 0 or 500 mg/kg-bw in "neutral oil"; tissues analysed 24 h after dosing (Thier et al. 1990) **Gene mutation (sex-linked recessive lethal)** **Negative:** in male *Drosophila melanogaster* (fruit flies) administered orally (diet) 0 or 2000 ppm for 72 h, adults then mated; injected 0 or 12 500 mg/L in 0.7% NaCl solution, 2- to 3-day-old males mated 24 h post-injection (Foureman et al. 1994)
Genotoxicity and related endpoints: *in vitro*	**Bacterial tests:** ***Ames test (mutagenicity)*** **Negative:** *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 strains (up to 3 mg/plate) with and without S9 (Mortelmans et al. 1986; NTP 1986) **Negative:** *Salmonella typhimurium* TA1535, TA1537, TA1538 strains (up to 1 mg/plate) with and without S9 (Hossack et al. 1978b) **Negative:** *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 strains (up to 5 mg/plate) with and without S9 (Hüls AG 1988a) ***DNA damage and repair assay*** **Negative:** *Salmonella typhimurium* TA1535/pSK 1002 *umu* test, at dose of 680.3 µg/mL per unit of bacterial density, with S9 (Ono et al. 1991) **Positive (weakly):** *Salmonella typhimurium* TA1535/pSK 1002 *umu* test, at dose of 680.3 µg/mL per unit of bacterial density, without S9 (Ono et al. 1991) **Negative:** *Salmonella typhimurium* strain TA1535/Psk1002 *umu* test, at dose up to 5000 µg/plate, with S9 (Yasunaga et al. 2004)

Endpoint	Lowest effect levels[1]/Results
	Positive: *Salmonella typhimurium* strain TA1535/Psk1002 *umu* test, at dose up to 5000 µg/plate, without S9 (Yasunaga et al. 2004) **Bacillus subtilis *recombination assay*** **Positive:** *Bacillus subtilis* H17 (arg−, trp−, recE+) and M45 (arg−, trp−, recE−), 2.18–5.93 µg/L; with S9, DNA damaging potential; without S9, reverse mutation (Matsui et al. 1989) **Mammalian cell tests:** ***Mutagenicity in mouse lymphoma cells*** **Positive:** in mouse lymphoma cells (L5178Y), 50–1600 µg/mL; in three duplicate experiments without added activation (−S9) (positive only at concentrations that reduced growth) (NTP 1986; Tennant et al. 1987; McGregor et al. 1988) **Negative:** mouse lymphoma cells (L5178Y) with S9 and without S9 in one of two laboratories (Honma et al. 1999a) **Positive:** in mouse lymphoma cells (L5178Y), without S9, dose-dependent positive response in one of two laboratories, but the maximum mutation frequency was <2 times the spontaneous one (Honma et al. 1999a) **Positive:** in mouse lymphoma cells (L5178Y), 0–1500 µg/mL, 24-h treatment, without S9. The incubation time was increased to 24 h to render the test system especially sensitive for the detection of clastogens and spindle poisons (Honma et al. 1999b) **Negative:** in mouse lymphoma assay (L5178Y), 119.6–1196 µg/mL (without S9); 81.9–818.8 µg/mL (with S9) (Microbiological Associates 1984b; O'Donoghue et al. 1988) ***Chromosomal aberrations*** **Negative:** Chinese hamster lung (CHL) cells, up to 1.250 mg/mL, in standard procedure, with and without S9 (Matsuoka et al. 1996) **Positive:** CHL cells, up to 1.750 mg/mL, in modified procedure, cells were treated for 6 h then for a further 18 h in fresh medium with and without S9 (Matsuoka et al. 1996) **Negative:** Chinese hamster ovary (CHO) cells, up to 1.600 mg/mL, with and without S9 (NTP 1986; Tennant et al. 1987; Gulati et al. 1989) ***Sister chromatid exchange*** **Positive:** CHO cells, up to 1.600 mg/mL, with and without S9; increased sister chromatid exchange only at cytotoxic concentrations detected after delayed harvest (6–13h additional culture time) (NTP 1986; Gulati et al. 1989) ***Unscheduled DNA synthesis*** **Negative:** primary liver cell cultures from adult male Sprague-Dawley rats; 0.005–0.4 µL/mL (Microbiological Associates 1984c; O'Donoghue et al. 1988) **Positive:** Hepatocytes from male Sprague-Dawley rats, up to 6.25 mM; induced DNA repair at non-cytotoxic doses (Selden et al. 1994) **Cell transformation assay** **Positive:** A31-1-13 clone of BALB/c-3T3 cells, 46–738 mg/L (Matthews et al. 1993)
Irritation	**Skin irritation** **Not irritating:** in 6 rabbits (male plus female), 0.5 mL per patch (corresponds to ~150 mg/kg-bw) undiluted, for 4 h, observed after occlusive and semi-occlusive application; no information available in secondary sources regarding strain, number of animals, exposure level (Potokar et al. 1985) **Irritating:** in 4 rabbits at 50, 200, 794 and 3160 mg/kg-bw (200 mg isophorone/kg-bw corresponds to about 0.5 mL undiluted substance) undiluted, occlusive for 24 h (Esso 1964) **Slightly irritating:** at dose of 200 mg/kg-bw, in group of 4 rabbits, treated with 50,

Endpoint	Lowest effect levels[1]/Results
	200, 794 or 3160 mg/kg (200 mg isophorone/kg-bw corresponds to about 0.5 mL undiluted substance), occlusive 24 h, reversible (Truhaut et al. 1972; Dutertre-Catella 1976) **Slightly irritating:** in 6 rabbits, undiluted 0.5 mL (body weight of rabbit not reported for unit conversion; approximately equivalent to 200 mg/kg-bw)) for 24 h (HRC 1979b) **Not irritating:** 6 male/female rabbits, New Zealand, undiluted, 0.5 mL per patch, 2 patches applied, equivalent to 300 mg/kg-bw for 1 h (Potokar et al. 1985) **Eye irritation** **Irritating:** in groups of 6 male and female rabbits, undiluted, 0.1 mL (equivalent to 40 mg/kg-bw), into conjunctival sac of left eye, closed for 30 s. Post-exposure period: 1, 4, 24 h; 2, 3, 4, 7, 10, 14 days; (Draize Test) scrambling and preening; phonation was noted in one animal (Esso 1964) **Irritating:** in 6 rabbits, undiluted 0.1 mL (equivalent to 40 mg/kg-bw) for 24 h, reversible (Truhaut et al. 1972; Dutertre-Catella 1976)
Sensitization	**Not sensitizing:** in 20 female guinea pigs, Albino, Bor: DHPW, 10% intracutaneous or undiluted occlusive epicutaneous in corn oil (according to OECD Test Guideline 406, positive control not required by 1981 guideline version) (Hüls AG 1988b)
Human volunteer studies	
Irritation	**Lowest LOEC** = 144 mg/m^3 (25 ppm) chamber concentration, based on eye, nose and throat irritation in 70% of 12 volunteers of both sexes exposed to isophorone vapours for 15 min (Silverman et al. 1946). Other studies: **LOEC** = 230 mg/m^3 (40 ppm), based on eye and nasal irritation in (number of volunteers affected not reported) groups of 11 or 12 subjects exposed for a few minutes in a small room. At higher concentrations (1150 and 2300 mg/m^3), a few complaints of nausea, headache, dizziness, faintness, inebriation and a feeling of suffocation were reported (Smyth and Seaton 1940). **LOEC** = 199 mg/m^3 (35 ppm) based on throat irritation in 1/6 (sex not specified) volunteers exposed to airborne isophorone for 7 min. Eye irritation was observed (number of volunteers affected not available) at 377 mg/m^3 (Esso 1965a).
Sensitization	**Not sensitizing:** in 10 humans, no further detail available (E.I. Du Pont de Nemours & Co. 1983)

[1] LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LOEC, lowest-observed-effect concentration; LOEL, lowest-observed-effect level; NOEL, no-observed-effect level.