Screening Assessment for the Challenge

Sulfuric Acid, Diethyl Ester
(Diethyl sulfate)

Chemical Abstracts Service Registry Number

64-67-5

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 Sulfuric acid, diethyl ester (Diethyl sulfate), Chemical Abstracts Service Registry Number 64-67-5. This substance was identified in the categorization of the Domestic Substances List as a high priority for action under the Challenge. Diethyl sulfate was identified as presenting an intermediate potential for exposure to individuals in Canada (IPE) and had been classified by other agencies on the basis of carcinogenicity and genotoxicity. Since the substance did not meet the ecological categorization criteria for bioaccumulation, persistence or inherent toxicity to aquatic organisms, the focus of this assessment relates to human health aspects.

According to data submitted in response to section 71 of CEPA 1999, no companies in Canada reported manufacturing diethyl sulfate in a quantity greater than or equal to the threshold of 100 kg for the 2006 calendar year. However, it was reported that approximately 1000 kg were imported into Canada for the same year. The responses to the section 71 request indicated that diethyl sulfate is mainly used in Canada as a chemical intermediate in the tissue paper industry. Based on information presented in the available scientific and technical literature, diethyl sulfate may be used as a chemical intermediate in the preparation of a variety of other substances and products, including dyes, fragrances, and quaternary ammonium salts used as surfactants or flocculants in water treatment. It may also be used as an ethylating agent to convert compounds such as phenols and thiols to their corresponding ethyl derivatives in the manufacture of commercial products such as sanitizers and organoclays.

Diethyl sulfate is not a naturally occurring compound. Emissions of diethyl sulfate into the environment are only expected to come from anthropogenic sources. The principal route of exposure for the general population would likely be through inhalation, based on its moderate vapour pressure. However, as diethyl sulfate is used principally in closed systems, releases are likely to be very low and would be rapidly hydrolyzed. Therefore, population exposure in the general environment is expected to be negligible. Consumer exposure to residual diethyl sulfate in products is also expected to be insignificant.

Based on the weight of evidence assessments of international and other national agencies and taking into consideration more recent data, the critical effect for the characterization of risks to human health for diethyl sulfate is carcinogenicity. Increased incidences of tumours (principally at the site of administration) were observed in rats and mice exposed via ingestion, dermal application or subcutaneous injection. Tumours were also observed in pups of rats exposed to diethyl sulfate during pregnancy. Diethyl sulfate was also consistently genotoxic in a range of *in vivo* and *in vitro* assays and is a strong DNA alkylating agent. While the mode of induction of tumours by diethyl sulfate has not been fully elucidated, it cannot be precluded that the tumours observed in experimental animals have resulted from direct interaction with genetic material.

On the basis of the carcinogenic potential of diethyl sulfate, for which there may be a probability of harm at any exposure level, it is concluded that diethyl sulfate is a substance that is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Diethyl sulfate does not meet the criteria for persistence or bioaccumulation potential set out in the *Persistence and Bioaccumulation Regulations*. On the basis of low ecological hazard and reported releases of diethyl sulfate, 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.

This substance will be included in the upcoming *Domestic Substances List* inventory update initiative. 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 the information available, it is concluded that diethyl sulfate meets one or more of the criteria set out in section 64 of CEPA 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 inherent toxicity 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 Sulfuric acid, diethyl ester (diethyl sulfate) was identified as a high priority for assessment of human health risk because it was considered to present IPE and had been classified by other agencies on the basis of carcinogenicity and genotoxocity. The Challenge for diethyl sulfate was published in the *Canada Gazette* on Nov. 17, 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.

Diethyl sulfate was determined to be a high priority for assessment with respect to risks to human health under CEPA 1999. However, it was not identified as a priority for assessment of potential ecological risks, based on the evaluation of persistence, potential for bioaccumulation and inherent toxicity to aquatic organisms conducted during the categorization of the Domestic Substances List. Therefore, this assessment focuses on information relevant to the evaluation of human health risks.

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.

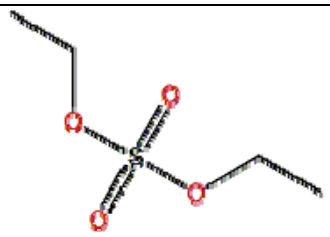
This screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to June 2008. 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 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 conclusion 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. The 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. Harlee Strauss (H.Strauss Associates, Inc.) and Dr.Glenn Talaska (U. of Cincinnati). Comments on these sections were also received from Gradient Corporation. The ecological portions of the assessment have also undergone external written peer review/consultation. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. Although 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 assessment is based are summarized below.

Substance Identity

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

Table 1. Substance identity for diethyl sulfate

Chemical Abstracts Service Registry Number (CAS RN)	**64-67-5**
Domestic Substances List (DSL) name	**Sulfuric acid, diethyl ester**
Inventory names[1]	*Diethyl sulfate* (ECL, PICCS) *Diethyl sulphate* (EINECS) *Sulfuric acid diethyl ester* (ECL) *Sulfuric acid, diethyl ester* (TSCA, ENCS, AICS, SWISS, PICCS, ASIA-PAC, NZIoC, ECL) *Ethyl sulfate* (TAIWAN)
Other names	*DES* *Ethyl sulfate (Et_2SO_4)* *NSC 56380* *UN 1594* *UN 1594 (DOT)*
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Esters
Major chemical sub-class	Dialkyl sulfate esters
Chemical formula	$C_4H_{10}O_4S$
Chemical structure	
SMILES[2]	O=S(=O)(OCC)OCC
Molecular mass	154.18 g/mol

[1] National Chemical Inventories (NCI). 2006: 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 (Swiss Giftliste 1 and Inventory of Notified New Substances); TSCA (Toxic Substances Control Act Chemical Substance Inventory); and TAIWAN (Taiwan).
[2] Simplified Molecular Line Input Entry System.

Physical and Chemical Properties

A summary of key physical and chemical properties for diethyl sulfate is presented in Table 2. At room temperature, diethyl sulfate is a colourless, oily liquid with a peppermint odour.

Table 2. Physical and chemical properties of diethyl sulfate

Property	Type	Value	Rating[1]	Reference
Melting point (ºC)	Experimental	-24.0 -25.0		CRC 2006 HSDB 2003
Boiling point (ºC)	Experimental	208.0		CRC 2006
Density (kg/m^3 at 25ºC)	Experimental	1172 (1.172 g/cm^3)		HSDB 2003
Vapour pressure (Pa)	Experimental	38.6 (0.29 mm Hg)	Moderate	Daubert and Danner 1991
	Experimental	28.26 (0.21 mm Hg)	Moderate	HSDB 2003
Henry's Law constant (Pa·m^3/mol)	Experimental	0.53 (5.22 x 10^{-6} atm m^3/mol)		SRC 1988 (calculated)
	Experimental	0.62 (6.14 x 10^{-6} atm m^3/mol)		SRC 2003
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	Experimental	1.14	Low	Hansch et al. 1995
Log K_{oc} (Organic carbon-water partition coefficient) (dimensionless)	Modeled	1.45[2] – 1.72[3]	Low	KOCWIN 2009
Water solubility (mg/L between 15 and 25ºC)	Experimental	7000	High	HSDB 2003

[1] qualitative relative rating of the physical-chemical parameter of a substance
[2] Koc estimated using the Molecular Connectivity Index (MCI) training and validation datasets
[3] Koc estimated from an EPISUITE derived logKow

Sources

Diethyl sulfate is not formed naturally in the environment; its presence results exclusively from anthropogenic sources. This chemical may enter the environment during its production and industrial use as an ethylating agent for a wide variety of organic functional groups and in the preparation of a wide variety of intermediates and end products (HSDB 2003). Diethyl sulfate may be released into the environment through various waste streams (HSDB 2003).

According to data submitted in response to section 71 of CEPA 1999, no companies in Canada reported manufacturing diethyl sulfate in a quantity greater than or equal to the threshold of 100 kg for the 2006 calendar year. However, it was reported that approximately 1000 kg were imported into Canada in that year (Environment Canada 2008).

Uses

According to submissions made under section 71 of CEPA 1999 (Environment Canada 2008), diethyl sulfate is mainly used as a chemical intermediate, especially in the paper industry. The substance can be found in residual amounts in chemical additives that are used as tissue softeners and as release technology aids to increase the absorbency of paper media.

Based on other available scientific and technical literature, diethyl sulfate is a powerful ethylating agent used in the preparation of a wide variety of intermediates, especially in the fields of dyes, agricultural chemicals, pharmaceuticals and textiles. Diethyl sulfate is commonly used in the manufacture of quaternary ammonium salts, which are used in textile applications as a finishing agent (NTP 2005), as fabric softeners in detergents and in dye and pigment manufacture to increase the affinity of the dye to the fibre (Dow 2006a). However, its use in the manufacture of quaternary ammonium salts to be used as fabric softener and its use in agricultural chemicals has not been identified in Canada. Quaternary ammonium salts are also used in hair care products such as shampoo and conditioner; germicides for disinfectants and sanitizers found in cleaners; drilling fluids and water cooling applications,as well as in the production of organoclays (Dow 2006a). Organoclays are used as viscosity modifiers in a broad range of products including drilling fluids, lubricants, oil-based paints, phase transfer catalysts, electroplating materials and emulsifying agents, which include asphalt additives and corrosion inhibitors (Dow 2006a).

Other applications of diethyl sulfate include its use as a dye-set agent in carbonless paper (NTP 2005) and as an accelerator in ethylene sulfation and sulfonations (HSDB 2003)

This chemical is currently not listed on Health Canada's Cosmetic Ingredient Hotlist as a prohibited substance in cosmetic products (Health Canada 2008a). In Canada, diethyl sulfate is not approved as a food additive nor has it been used in food packaging materials

and incidental additives used in food plants (2009 email from Food Directorate to Existing Substance Bureau, Health Canada, unreferenced). This substance is not registered as an active ingredient or a formulant in pest control products (PMRA 2008). Diethyl sulfate is not included in the Drug Product Database, Natural Health Products Ingredients Database nor the Licensed NHP Database, therefore it is not used in Canada in pharmaceutical, natural health products or veterinary drugs. Diethyl sulphate has not been identified as being present in these products during initial screening exercise. The *Controlled Products Regulations* established under the *Hazardous Products Act* requires this substance to be disclosed on the Material Safety Data Sheet that must accompany workplace chemicals when it is present at a concentration of 0.1% or greater, as specified on the Ingredient Disclosure List (Health Canada 2008b).

Releases to the Environment

Diethyl sulfate is not manufactured in Canada, and domestic supply is met by imports. Emissions of diethyl sulfate into the environment may occur during its use as an ethylating agent in the preparation of a wide variety of intermediates and products. Production and processing of diethyl sulfate normally occurs in closed systems, and no monitoring data on emissions are available.

Fugitive emissions or venting during the handling, transport or storage of diethyl sulfate could also be sources of release to the atmosphere. Direct releases of diethyl sulfate to the environment are unlikely, since it is mainly used as a chemical intermediate, and residuals from production would be rapidly hydrolyzed.

Under the National Pollutants Release Inventory (NPRI), there were no reportable releases of diethyl sulfate in 2006 or in previous years (NPRI 2006). In recent information gathered under a CEPA 1999 section 71 notice with respect to diethyl sulfate, no companies reported releasing this substance in 2006 (Environment Canada 2008).

Environmental Fate

Based on the results of Level III fugacity modelling (Table 3), diethyl sulfate will remain predominantly in the compartment into which it is released. Based on its uses and potential releases and its moderate vapour pressure, diethyl sulfate is expected to exist principally as a vapour in ambient air. Degradation in the atmosphere by moisture is rapid, with a half-life of less than 1 day (HSDB 2003).

Table 3. Results of the Level III fugacity modelling (EQC 2003) [1]

	Fraction of substance partitioning to each medium (%)			
Substance released to:	**Air**	**Water**	**Soil**	**Sediment**
Air (100%)	99.75	0.14	0.11	0
Water (100%)	0.10	99.9	0	0
Soil (100%)	0.34	0.16	99.5	0
Air, water, soil (33% each)	77.6	11.2	11.2	0

1. Identical hydrolysis rate values were used for the water, soil and sediment compartments.

Diethyl sulfate is highly soluble in water where it is expected to hydrolyze rapidly (see Table 4); monoethyl sulfate (Dow 2006ab, Erhardt 2006), ethanol and sulfuric acid have been identified as hydrolysis products (HSDB 2003). Diethyl sulfate is not expected to adsorb significantly to suspended solids or sediments, based on its low log K_{oc}. Volatilization is not expected to be an important removal process from water because of the rapid hydrolysis rate (HSDB 2003), and as indicated by its low Henry's Law constant (0.53-0.62 Pa m^3/mol). Although diethyl sulfate is expected to be relatively mobile when released to soil, based on its low log K_{oc} value, fugacity modelling indicates that most of the substance will remain in soil, with hydrolysis expected to be the dominant removal process in moist soils (HSDB 2003).

Persistence and Bioaccumulation Potential

Environmental Persistence

The empirical data available for persistence of diethyl sulfate are presented in Table 4.

Table 4. Empirical data for persistence of diethyl sulfate

Medium	Fate process	Degradation value	Degradation endpoint/units	Reference
Air	Photooxidation	4.4	Half-life (days, calculated)[a]	Japar et al. 1990
Air	Hydrolysis	≥ 16.7	Half-life (hours, calculated)[b]	Japar et al. 1990
Water	Ready-biodegradation	89	Biodegradation (%)	MITI 1992
Water	Hydrolysis	1.7	Half-life (hours)	Robertson and Sugamori 1966, HSDB 2003
		1.9	Half-life (hours)	Dow 2006 ab; Erhardt 2006

[a] Calculated from an atmospheric lifetime of 6.4 days for clean tropospheric conditions ([OH] = 1 x 10^6 cm^{-3}), [OH] reaction rate constant of 1.8 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1} (Japar et al. 1990).
[b] Calculated from an atmospheric lifetime of ≥ 1 days for clean tropospheric conditions ([H_2O] = 15 Torr), [H_2O] reaction rate constant ≤2.3 x 10^{-23} cm^3 $molecule^{-1}$ s^{-1} (Japar et al. 1990).

In air, degradation via photochemically produced hydroxyl radicals is not expected to be a major fate process for diethyl sulfate, based on a calculated half-life value of 4.4 days

(Japar et al. 1990). The substance is not expected to degrade via direct photolysis. However, a potential for rapid degradation via hydrolysis is suggested by a calculated half-life value ≥ 16.7 hours, which suggests that diethyl sulfate is not persistent in air (Japar et al. 1990) and is not subject to long range transport. In addition, an empirical hydrolysis half-life in air of less than 1 hour for the analogue dimethyl sulfate has been reported by Lee et al. (1980). Thus, diethyl sulfate is considered not persistent in air according to the half-life criterion of ≥ 2 days specified in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Diethyl sulfate is likely to be rapidly transformed and hydrolyzed in water, based on the experimental hydrolysis half-life values of 1.7 hours (Robertson and Sugamori 1966; HSDB 2003) and 1.9 hours (Dow 2006ab, Erhardt 2006). Primary hydrolysis of diethyl sulfate may result in the formation of monoethyl sulfate and ethanol (Dow 2006ab, Erhardt 2006) while the ultimate hydrolysis of diethyl sulfate will produce ethanol and sulfuric acid (HSDB 2003). The results of an experimental ready-biodegradation study show that 89% of diethyl sulfate biodegrades in 28 days (MITI 1992), indicating that the substance undergoes rapid ultimate and primary biodegradation. Thus, based on the empirical biodegradation and hydrolysis data, it is concluded that diethyl sulfate is not persistent in water according to the criterion specified in the *Persistence and Bioaccumulation Regulations* (half-life in water ≥ 182 days).

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al 1995), the half-life in soil is also <182 days and the half-life in sediments is < 365 days. Thus, diethyl sulfate is not expected to be persistent in soil and sediment according to the criteria specified in the *Persistence and Bioaccumulation Regulations* (half-life in soil ≥ 182 days and half-life in sediment ≥ 365 days) (Canada 2000).

Potential for Bioaccumulation

The experimental log K_{ow} value for diethyl sulfate (Table 2) suggests that this chemical has a low potential to bioaccumulate in the environment.

Since no experimental bioaccumulation factor (BAF) and/or bioconcentration factor (BCF) data for diethyl sulfate were available, a model approach was applied. The modelled BAF value of 1.8 L/kg indicates that diethyl sulfate does not have the potential to bioaccumulate in the environment. Modelled BCF of 1.5 to 15.9 L/kg (Table 5) support the low bioaccumulation potential of this substance.

Considering their physical and chemical properties the principal hydrolysis products, monoethyl sulfate, ethanol and sulfuric acid are also expected to have a low potential to bioaccumulate BCFWIN 2000).

Table 5. BAF and BCF predictions for diethyl sulfate in fish with a default of no metabolism

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BAF	1.8	Gobas BAF middle trophic level (Arnot and Gobas 2003)
Fish	BCF	1.7	Gobas BCF middle trophic level (Arnot and Gobas 2003)
Fish	BCF	4.3	ACD/pKaDB 2005
Fish	BCF	15.9	Baseline BCF model without mitigating factors (Dimitrov et al. 2005)
Fish	BCF	9.5	Baseline BCF model with mitigating factors (Dimitrov et al. 2005)
Fish	BCF	1.5	BCFWIN 2000

Based on the available kinetic based and other modelled values, diethyl sulfate does not meet the bioaccumulation criteria (BCF or BAF ≥ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

As indicated earlier, diethyl sulfate does not meet the persistence or bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Although no experimental toxicity values were identified for this substance, diethyl sulfate is not expected to cause significant harm to aquatic organisms at low concentrations (LC/EC_{50}s are expected to be > 1.0 mg/L), based on experimental data for its analogue, dimethyl sulfate. Indeed, a European Union assessment of dimethyl sulfate (EURAR 2002) cites empirical toxicity values ranging from a 96-hr LC_{50} for fish of 14 mg/L (Hoechst 1981), to a 48-hr EC_{50} for daphnia of 17 mg/L (Hoechst 1990), to a 72-hr EC_{50} for algae of 46.9 mg/L (Hoechst 1988). In addition, its hydrolysis rate in water (half life < 1.7-1.9 h) is rapid. Finally, considering the hydrolysis products, monoethyl sulfate, ethanol and sulfuric acid, empirical toxicity data for these compounds indicate that neither would be expected to cause significant harm to aquatic organisms at low concentrations (acute LC/EC_{50}s are expected to be > 1 mg/L) (Dow 2006ab, Marino et al. 2005ab, Hancock et al. 2005, El Jay, 1996, Majewski 1978 and Environment Canada 1984).

Since the quantity of diethyl sulfate imported into or used in Canada (~1000 kg) (Environment Canada 2007) is not exceptionally large and is mostly used in closed systems as a chemical intermediate, releases into the Canadian environment are expected to be very low. Because of this and given the likelihood of rapid hydrolysis, significant exposure of organisms in environmental media is considered unlikely.

Therefore, based on available information, diethyl sulfate is unlikely to cause ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

Experimental data for ecotoxicity and degradation were limited, and no relevant experimental data for bioaccumulation were identified. Estimation of the bioaccumulation properties therefore relied primarily on QSAR models. While there are uncertainties associated with the use of QSAR models to estimate chemical and biological characteristics, the approaches used are considered to yield credible results based on the chemical structure of the substance and additional information obtained for the analogue dimethyl sulfate (EURAR 2002)..

It is also noted that this evaluation focused primarily on data on toxicity to organisms in the pelagic aquatic environment. Although the release and partitioning of this substance in the environment could result in the exposure of organisms in other media (air, soil), no relevant toxicity data have been identified.

Potential to Cause Harm to Human Health

Exposure Assessment

No measured concentrations of diethyl sulfate in air in Canada or elsewhere were identified. Although there have been studies in which the presence of diethyl sulfate in indoor and outdoor air has been investigated, no quantitative data have been reported. Furthermore, there are no monitoring data in water or soil from which upper bounding exposure can be estimated. However, as diethyl sulfate is rapidly hydrolyzed, levels of this substance in environmental media are expected to be negligible.

Modelled estimates based on the industrial releases to the atmosphere reported under the recent section 71 notice (Environment Canada 2008) predict that concentrations of diethyl sulfate in air are low, at approximately 0.7 ng/m^3 (ChemCAN 2003). Predicted concentrations for water and soil are also very low (i.e., much less than 10^{-3} ng/L and 10^{-3} ng/g, respectively) (ChemCAN 2003). Likewise, food chain accumulation is unlikely based on a low log K_{ow} value; therefore, concentrations in foods are not expected to be significant.

Potential human exposure may occur as a result of residual diethyl sulfate in formulated end products (e.g., textiles, dyes and pharmaceuticals). However, no data on residuals were identified. Based upon the information provided by companies in Canada under the recent section 71 notice issued in accordance with CEPA 1999, diethyl sulfate is mainly used as an intermediate and has no direct use in consumer products. The substance was also not included in the U.S. Household Products Database (HPD 2008). Consumer exposure is therefore expected to be negligible.

Confidence in the quantitative estimates of exposure to diethyl sulfate in environmental media is considered to be very low to low, as the estimates are based on modelling. However, confidence is high that exposure to the substance by the general population is very limited, in light of the indication that it is not released to the general environment in Canada, and because of its very reactive nature.

Health Effects Assessment

An overview of the toxicological database for diethyl sulfate is presented in Appendix 1.

On the basis of investigations in experimental animals, diethyl sulfate has been classified by the International Agency for Research on Cancer (IARC) as a Group 2A carcinogen, “probably carcinogenic to humans” (IARC 1999). The European Commission has classified diethyl sulfate as a Category 2 carcinogen, “should be regarded as if carcinogenic to man” (ESIS 2008), while the National Toxicology Program (NTP) considers that this substance is “reasonably anticipated to be a human carcinogen” (NTP

2005). The available studies for diethyl sulfate that have been considered in this assessment are summarized below and in more detail in Appendix 1.

Diethyl sulfate has induced tumours at multiple sites in rodents exposed via dermal contact, oral ingestion or subcutaneous injection; no adequate inhalation carcinogenicity bioassays have been identified for this substance. Male C3H/HeJ mice administered undiluted diethyl sulfate via the dermal route (7.4 mg diethyl sulfate/mouse/application, 3 applications/week) for up to 22 months developed skin tumours, including squamous cell carcinomas or fibrosarcomas (21 of 27 exposed mice versus none in controls). An increased incidence of squamous cell carcinomas of the stomach was also observed in exposed mice; however the study authors speculated that these tumours likely resulted from oral ingestion of diethyl sulfate during grooming (Union Carbide 1979). Druckrey et al. (1970) examined the carcinogenic potential of diethyl sulfate in rats following oral, subcutaneous or transplacental exposure. For the oral route, BD rats administered 25 or 50 mg/kg-bw of diethyl sulfate once per week by gavage for 81 weeks were reported to have low incidences (1/12 in each dose group) of squamous cell carcinomas of the forestomach. Benign papillomas were also observed in exposed rats (6/24 in both dose groups combined). In the same study, pregnant female BD rats were administered a single subcutaneous injection of diethyl sulfate (85 mg/kg-bw) on day 15 of gestation. A low incidence (2/30) of malignant tumours of the nervous system (neurimomas) was reported in surviving pups of exposed dams. The authors noted that spontaneous tumours of this type had not been observed in historical control BD rats. Subcutaneous injections of diethyl sulfate (25 or 50 mg/kg-bw) once per week to BD rats for 49 weeks also induced tumours at the site of injection, with metastasis to the lungs at the higher dose. While these studies were all limited by lack of controls, small number of dose groups, small group sizes, limited pathological examination or high mortality rates, collectively they provide evidence of the carcinogenicity of diethyl sulfate in experimental animals.

Diethyl sulfate has also been consistently genotoxic in multiple *in vivo* and *in vitro* assays. Diethyl sulfate has been classified by the European Commission as a Mutagen Category 2 (substances which should be regarded as if they are mutagenic to man; risk phrase R46: may cause heritable genetic damage) (ESIS 2008). The IARC Working Group also concluded that "diethyl sulfate is a strong direct-acting alkylating agent which ethylates DNA" and that, as a result, "it is genotoxic in virtually all test systems examined, including potent effects in somatic and germ cells of mammals exposed in vivo" (IARC 1999). A detailed overview of the results of the available genotoxicity studies is presented in Appendix 1 and briefly summarized below.

Diethyl sulfate was clastogenic in all identified *in vivo* assays in several strains of rats and mice, inducing micronuclei, chromosome aberrations and aneuploidy. Diethyl sulfate also caused DNA damage (strand fragmentation) in multiple tissues of mice and rats. In addition, diethyl sulfate was shown to be a direct alkylating agent *in vivo* by measurement of DNA adducts (N7-ethylguanine) in multiple tissues of mice following intraperitoneal administration. While mixed results for genotoxicity in germ cells of rodents exposed *in vivo* (dominant lethal test, specific locus test and spermatocyte clastogenicity) have been reported in the literature, both IARC (1999) and the European

Commission (ESIS 2008) considered diethyl sulfate to be genotoxic in germ cells of mammals. An inconclusive result was reported for an *in vivo* assay of somatic cell mutations in mice (mouse spot test). In *Drosophila*, although one negative result was reported for heritable translocations, various positive mutagenic effects of diethyl sulfate were observed. In *in vitro* investigations, diethyl sulfate consistently tested positive in a range of assays for clastogenicity and mutagenicity in cultured mammalian cells and for mutagenicity in several strains of bacteria.

Information on the potential carcinogenicity and mutagenicity of diethyl sulfate in humans is limited to two retrospective cohort and three case-control studies. Although Lynch et al. (1979) reported an increased relative risk of laryngeal cancers (SMR = 5.04) in a cohort of 335 mainly caucasian male workers at an ethanol and isopropanol plant and concluded that "it appears likely from the data that diethyl sulfate was the primary carcinogen," The authors noted that this conclusion was complicated by potential co-exposure to several suspect carcinogens. In a follow-up nested case-control study in this group of workers, a statistically significant association was reported between the incidence of upper respiratory tract cancers (OR = 5.2) or laryngeal cancers (OR = 13.4) and high exposure to sulphuric acid (Soskolne et al. 1984). The IARC working group concluded that these data "support the role of sulphuric acid independent of dialkyl sulfates; however, it does not preclude a role for dialkyl sulfates" (IARC 1992). In another retrospective cohort study, investigators reported a significant increase in the incidence of lymphosarcoma and reticulosarcoma in 538 workers in one of two ethanol and isopropanol production plants (SMR = 5.60), while no such increase was observed in 493 workers at the other plant (Teta et al. 1992). In two separate case-control studies of workers from the same plastics plant, no significant association between occupational exposures to diethyl sulfate and cases of brain tumours was reported (Leffingwell et al. 1983, Austin and Schnatter 1983). The IARC Working Group noted the lack of empirical exposure data for diethyl sulfate in the above studies and the possibility of co-exposure to other potentially carcinogenic substances, and considered the epidemiology data to provide "inadequate evidence for the carcinogenicity in humans of diethyl sulfate" (IARC 1999)

Although a thorough analysis of the potential mode of action for induction of tumours by diethyl sulfate is beyond the scope of this screening assessment, both IARC and NTP have stated that diethyl sulfate is a strong alkylating agent and has the potential to react with macromolecules such as nucleic acids (IARC 1999, NTP 2005). Indeed, ethylation of DNA (7-ethylguanine, O^6-ethylguanine adducts) has been observed in the liver, bone marrow and testes of mice exposed to diethyl sulfate via a single intraperitoneal injection (Van Zeeland et al. 1990).

The available database on non-cancer effects associated with exposure to diethyl sulfate is very limited. Diethyl sulfate caused edema and necrosis in the skin of rabbits following acute dermal exposure (Union Carbide 1982, Smyth et al. 1949), while rabbits displayed corneal injury in an eye irritation test (Smyth et al. 1949). Pulmonary edema and hemorrhagic damage to the intestinal mucosa were reported in rats exposed to 350 mg/kg-bw diethyl sulfate (the LD_{50}) in an acute oral study (Druckrey et al. 1970).

The only repeated-dose study identified in which non-neoplastic effects were reported was the chronic skin painting study in mice administered 7.4 mg diethyl sulfate/animal, 3 times per week for up to 22 months (Union Carbide 1979). Other than the skin and stomach tumours discussed above, the authors reported that there was no significantly increased frequency of any lesions in tissues of exposed mice (Union Carbide 1979). In female rats administered the substance via intraperitoneal injection at 150 mg/kg-bw, 4 hours pre-mating, there was a statistically significant increase in pre-implantation loss (Generoso et al. 1991). In addition, reproductive effects were observed in other studies at approximately the same dose range (170 mg/kg-bw and 180 mg/kg-bw respectively) in mice (Bishop et al. 1997).

The confidence in the toxicity database in experimental animals is considered to be low to moderate. Although data were identified for acute, repeat dose, reproductive and developmental toxicity, carcinogenicity and genotoxicity, the repeat dose studies are limited and sufficient human epidemiology data are not available. There is also uncertainty regarding the mode of induction of tumours and the levels at which noncancer effects are induced. However, confidence is higher that diethyl sulfate is carcinogenic and genotoxic in experimental animals.

Characterization of Risk to Human Health

Based on the weight of evidence assessments of several international agencies (IARC, European Commission and NTP), and taking into consideration more recent data, a critical effect for characterization of risk to human health for diethyl sulfate is carcinogenicity, for which a mode of induction involving direct interaction with genetic material cannot be precluded. Although there are limitations to many of the individual studies in experimental animals, collectively the evidence is considered sufficient. Diethyl sulfate is a strong direct alkylating agent that has induced tumours in rats and mice (including in pups exposed *in utero*) and has consistently induced genotoxic effects in a range of *in vivo* and *in vitro* assays. While the results of available epidemiological studies do not provide any conclusive evidence of carcinogenicity in humans, the observation of respiratory tract tumours in exposed workers is consistent with the evidence from studies in rodents in which diethyl sulfate induced tumours at the site of contact.

The available database on non-cancer effects induced by diethyl sulfate is very limited. In particular, no adequate data were identified on effects observed in epidemiological studies or in laboratory animals exposed via inhalation (the likely most relevant route of exposure for the general population) to permit derivation of margins of exposure. However, based on the conservative upper bounding estimate of levels in ambient air in Canada and the limited toxicological data available for other routes of exposure, such margins would likely be in the range of many orders of magnitude, and would likely be considered adequately protective for non-cancer effects.

Uncertainties in Evaluation of Risk to Human Health

The scope of this screening assessment does not take into account possible differences between humans and experimental species in sensitivity to effects induced by diethyl sulfate, particularly in light of the limited data available for humans. However, since diethyl sulfate is a direct acting genotoxicant, it is likely that the tumours observed in experimental animals are relevant to humans. In addition, the mechanism of tumour induction has not been fully elucidated, although the data suggest that the strong alkylation potential of diethyl sulfate may play a role. Furthermore, there is uncertainty regarding the precise magnitude of exposure to diethyl sulfate in the general environment, although exposures are expected to be very low. As well, available sources of information indicate that diethyl sulfate is not used directly in products that result in exposure of the general population. Therefore, since the estimate of exposure presented in this assessment is conservative, confidence is high that actual exposure from all potential sources would not exceed these estimates.

Conclusion

Based on the available information, it is concluded that diethyl sulfate 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.

On the basis of the carcinogenicity of diethyl sulfate, for which there may be a probability of harm at any level of exposure, it is concluded that diethyl sulfate is a substance that is entering or may enter 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 diethyl sulfate does not meet the criteria in paragraph 64(a) and 64(b) of CEPA 1999, but it does meet the criterion in paragraph 64(c) of CEPA 1999. Additionally, diethyl sulfate does not meet criteria for persistence and bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations.*

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Appendix 1: Summary of Health Effects Information for Diethyl Sulfate (CAS RN 64-67-5)

Endpoint	Lowest effect levels[ab]/Results
Acute toxicity	**Lowest oral LD_{50}** = 350 mg/kg-bw in rats (Druckrey et al. 1970). Pulmonary edema and hemorrhagic damage to the intestinal mucosa were reported in rats following acute oral exposure (Druckrey et al. 1970). [Additional acute oral studies: Smyth et al. 1949] **Lowest inhalation LC_{50}** = between 1580 and 3160 mg/m^3 in rats (Smyth et al. 1949).[2] After 4 hour exposure, 0/6 rats dead at exposure of 250 ppm (1580 mg/m^3) and 6/6 dead at exposure of 500 ppm (3160 mg/m^3) (Smyth et al. 1949) Clinical observations were not reported in this study. [Additional acute inhalation studies: Dupont 1994] **Lowest dermal LD_{50}** = 706 mg/kg-bw in rabbits (Union Carbide 1951). Diethyl sulfate was reported to be irritating in rabbits and to cause both necrosis (Smyth et al. 1949) and edema (Union Carbide 1982). No irritation was reported in guinea pigs following dermal application (Dupont 1994). [Additional acute dermal studies: none identified] Other effects: Diethyl sulfate was reported to be irritating and to cause corneal injury in rabbits (Smyth et al. 1949) and in guinea pigs (Dupont 1994) following administration to the eye.
Short-term toxicity [repeated-dose]	No data identified
Subchronic toxicity	No data identified
Chronic toxicity/ carcinogenicity	**Non-neoplastic endpoints** *Oral:* In the chronic oral study by Druckrey et al. (see entry above for details) there was no reporting of non-neoplastic effects provided (Druckrey et al. 1970). *Dermal:* In the chronic skin painting study by Union Carbide (see entry below for details), male C3H/HeJ mice (40/group) were exposed on the clipped skin of back to undiluted diethyl sulfate at 7.4 mg per application per mouse (equivalent to 247 mg/kg-bw per application),[3] 3 times per week for up to 22 months (100% mortality by 23 months). The gross and histopathological examination of various organs did not show any apparent exposure-related differences between the diethyl sulfate and control groups. The study authors considered any slight differences observed to be of "minimal importance" and stated that "there was no increased frequency of these minor lesions in the diethyl sulfate-treated mice" (Union Carbide 1979). [Additional chronic toxicity studies: Druckrey et al. 1970]

[2] After 4 hour exposure, 0/6 rats dead at 250 ppm (1580 mg/m^3) and 6/6 dead at 500 ppm (3160 mg/m^3) (Smyth et al. 1949)

[3] Dose conversion of 247 mg/kg-bw = 7.4 mg / 0.03 kg-bw for a mouse, mouse weight from Health Canada 1994)

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Endpoint	Lowest effect levels[ab]/Results
	Neoplastic endpoints: *Inhalation:* N/A (no inhalation repeated exposure studies were identified) *Oral:* BD rats (12/group) were exposed (gavage) to diethyl sulfate at 25 or 50 mg/kg-bw/day, 1day/week for 81 weeks. A squamous cell carinoma of the forestomach was found in each exposed group (1/12 in each dose group) while some animals of the exposed groups (6/24, distribution among dose groups not indicated) were reported to have "a number of benign papillomas" (Druckrey et al. 1970). [The IARC Working Group noted the small number of animals used and lack of concurrent control group as limitations for this study (IARC 1992)]. *Dermal:* Male C3H/HeJ mice (40/group) were exposed dermally (clipped skin of back) to undiluted diethyl sulfate at 7.4 mg per application per mouse (equivalent to 247 mg/kg-bw per application)[4] , 3 times per week for up to 22 months (100% mortality by 23 months). The control group was exposed to acetone at 12.6 mg per mouse, 3 times per week for lifetime (up to 27 months) and the animals in the positive control group were exposed to methylcholanthrene (as a 0.2% diluted in benzene) at an average dose of 0.033 mg per mouse, 3 times per week for 6 months (100% mortality in the positive control group by 6 months). All animals used for this assay were between 8 and 9 weeks of age upon study initiation and were examined monthly. For the diethyl sulfate exposed group, malignant skin neoplasms were observed in 21/27 surviving mice after 22 months of exposure (27/40 mice alive in diethyl sulfate group at appearance of the first tumour). This tumour incidence is compared to 36/39 surviving mice in the positive control group after 6 months, while no tumours were observed in the vehicle control group after 27 months. Microscopic examination of a subset of the tumours from the diethyl sulfate and positive control groups indicated the tumour types to be squamous cell carcinomas or fibrosarcomas (Union Carbide 1979). *Other routes:* BD rats (12/group) received subcutaneous injections of diethyl sulfate at 25 or 50 mg/kg-bw/day (in arachis oil) once/week for 49 weeks. In the high dose group, 11 surviving rats were reported to have developed local tumours at the injection site (3 spindle cell sarcomas, 3 fibrosarcomas, 3 myosarcomas, 1 polymorphocellular sarcoma and 1 glandular carcinoma). Two of the 11 surviving rats also had metastatis to the lungs. In the low dose rats, 6/12 were reported to have local tumours at the injection site (3 fibrosarcomas, 2 spindle-cell sarcomas and 1 myosarcoma) (Druckrey et al. 1970, also cited as Preussman 1968). [The IARC Working Group noted the lack of concurrent control group for this study although no local tumours were observed in historical vehicle controls (IARC 1992)] Three pregnant female BD rats received a single subcutaneous injection of diethyl sulfate (85 mg/kg-bw, unknown vehicle oil) on day 15 of gestation. The pups from the treated dams were observed until their death. Malignant tumours of the nervous system (neurinomas) were reported in 2/30 surviving pups. The authors noted that the incidence of spontaneous tumours of this type was reported to be low in historical control BD rats (Druckrey et al. 1970).

[4] Dose conversion of 247 mg/kg-bw = 7.4 mg / 0.03 kg-bw for a mouse, mouse body weight from Health Canada 1994)

Appendix 1: Summary of Health Effects Information for Diethyl Sulfate (CAS RN 64-67-5)

Endpoint	Lowest effect levels[ab]/Results
	[The IARC Working Group noted the small number of animals tested and lack of concurrent control group as limitations to this study (IARC 1992)]
Reproductive toxicity	**LOEL** = 150 mg/kg-bw: Statistically significant ($p<0.01$) [increases in post-implantation loss were observed in (C3HxC57BL)F_1 mice exposed via intraperitoneal (ip) injection 1 hour post-mating to unexposed males. No significant effects were noted in rats exposed to 150 mg/kg-bw (single dose) 4 hours pre-mating, or at 6, 9, or 25 hours post-mating.] (Generoso et al. 1991) [Additional studies: Bishop et al. 1997, Seiler 1977]
Developmental toxicity	**LOEL** = 180 mg/kg-bw: Statistically significant ($p<0.01$) [Increases in post-implantation mortality and increased incidence of malformations in the postnatal pups of (C3HxC57BL)F_1 mice exposed via ip injection to 180 mg/kg-bw (single dose) 4 hours pre-mating, or 1, 6, 9, or 25 hours post-mating with unwexposed males] (Generoso et al. 1991) [Additional studies: Bishop et al. 1997, Druckrey et al. 1970]
Genotoxicity and related endpoints: *in vivo*	**Clastogenicity:** From the results of 3 independent tests in the BDF1 mouse (ip injection), diethyl sulfate was reported to be positive for induction of micronuclei in peripheral blood cells and bone marrow cells (Morita et al. 1997). From the results of 2 independent tests in the SD rat (ip), diethyl sulfate was reported to be positive for induction of micronuclei in peripheral blood cells and bone marrow cells (Wakata et al. 1998). Positive in male ddY mice (ip), peripheral blood reticulocytes for micronuclei induction (Asita et al. 1992). Positive in male MS/Ae strain mice (ip), peripheral blood reticulocytes for micronuclei induction (Higashikuni and Sutou 1995). Positive in NMRI mouse (ip) embryo cells for chromosome aberrations (Braun et al. 1986). Positive in CBA/CaLacY mouse (ip) bone marrow cells for aneuploidy (Surkova and Malashenko 1974). **Genotoxicity in somatic cells:** Positive/negative/inconclusive in C57BL/6 Jena XT mice (ip) for spot tests (Braun et al. 1984). This study was reported to be inconclusive by IARC (1999). **Genotoxicity in germ cells:** Positive in (101/El x C3H/E1) F_1 mice (ip) for specific locus test (Ehling and Neuhauser-Klaus 1988). Positive/negative/inconclusive (interpretation varied in secondary sources) in C3H male and YT female mice for specific locus test (Malashenko 1976). Positive/negative/inconclusive (interpretation varied in secondary sources) in

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Endpoint	Lowest effect levels[ab]/Results
	C57BL/10ScSnEg D (H-2d) mice for specific locus test (Egorov and Blandova 1972). Negative in BALB/c mice for meiotic chromosome changes in spermatocytes (Léonard et al. 1971). Positive in (101/El x C3H/E1)F_1 male mice (ip, mated with unexposed females) for dominant lethal test (Ehling and Neuhauser-Klaus 1988). Positive/negative/inconclusive (interpretation varied in secondary sources) in mice (ip, unknown strain) for dominant lethal test (Malashenko and Egorov 1968). Inconclusive in mice (intrascrotal C3H males, mated with untreated CBA females) for dominant lethal test (Malashenko and Egorov 1968). Inconclusive in C57BL mice (intrascrotal, mated with CBA females) for dominant lethal test (Malashenko 1971). **DNA damage or repair:** Positive for the comet assay in ddY mouse (ip), measured in nuclei isolated from cells of various tissues (stomach, colon, kidney, liver, bladder, lung, brain, bone marrow) (Tsuda et al. 2000). Positive for DNA fragmentation (by alkaline elution) in albino white rats (intravenous) (Robbiano and Brambilla 1987). Positive for DNA adducts (N7-ethylguanine) in male (101/El x C3H/E1)F_1 mice (ip), detected in various tissues (liver, germ cells, testes, tumuli, bone marrow) (Van Zeeland et al. 1990). **Genotoxicity in other species:** Positive in *Pleurodeles waltl* for micronucei induction (Jaylet et al. 1986). Positive in *Drosophila melanogaster* for genetic crossing-over or recombination (Pelecanos 1966). Positive in *Drosophila melanogaster* for sex-linked recessive lethal mutations (Vogel 1989). Positive in *Drosophila melanogaster* for sex-linked recessive lethal mutations (Abraham et al. 1979) Negative in *Drosophila melanogaster* for heritable translocations (Pelecanos 1966). Positive in *Drosophila melanogaster* for recessive lethal mutations in female germ cells (Hernando et al. 2004). Positive in *Drosophila melanogaster* for recessive lethal mutations in female germ cells (Sierra et al. 1999).

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Endpoint	Lowest effect levels[ab]/Results
Genotoxicity and related endpoints: *in vitro*	**Clastogenicity:** Positive in human lymphocytes cells for micronuclei inductions (De Ferrari et al. 1988). Positive in Chinese hamster ovary fibroblasts for micronuclei inductions (Campagna et al. 2003). Positive in Chinese hamster lung V79 cells for micronuclei inductions (Bonatti et al. 1986). Positive in Chinese hamster lung V79 cells for micronuclei inductions (De Ferrari et al. 1988). Positive in Chinese hamster lung V79 cells for micronuclei inductions (Nüsse et al. 1989). Positive in human lymphocytes cells for aneuploidy (De Ferrari et al. 1988). Positive in Chinese hamster ovary CHO cells for chromosome aberrations (Asita 1989). Positive in Chinese hamster lung V79 cells sister chromatid exchanges (Nishi et al. 1984). **Mutagenicity in mammalian cell lines:** Positive in Chinese hamster ovary CHO cells for gene mutations (Couch et al. 1978). Positive in Chinese hamster ovary CHO cells for gene mutations on the hgprt locus (Bignami et al. 1988). Positive in Chinese hamster ovary CHO cells for gene mutations on the Na/K ATPase locus (Bignami et al. 1988). Positive in Chinese hamster lung V79 cells for gene mutations on the hgprt locus (Mohn and Van Zeeland 1985). Positive in Chinese hamster lung V79 cells for gene mutations on the hgprt locus (Nishi et al. 1984). **DNA damage or repair:** Positive in human lymphocytes and granulocytes for DNA ethyl-adducts and breaks (Schutte et al. 1988). Positive in Chinese hamster ovary CHO cells for DNA single strand breaks (Abbondandolo et al.. 1982). Positive in Chinese hamster ovary CHO cells for DNA single strand breaks (Dogliotti et al. 1984). Positive in rat primary hepatocytes for unscheduled DNA synthesis (Probst et al. 1981). Positive in h2E1/OR cells for DNA adducts (LePla et al. 2006).

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Endpoint	Lowest effect levels[ab]/Results
	Positive in isolated calf thymus DNA for DNA methylation (Singh et al. 2005). **Mutagenicity in bacteria:** Positive *in S. typhimurium* strains TA88, 90, 97, 1537, 2637, 3243, hisC3076 and negative in strains TA1537, hisD3052 for mutations (Levin et al. 1982). Positive in *S. typhimurium* (TS1121, 1157) for mutations (Hoffmann et al. 1988). Positive in *S. typhimurium* strain TA100 and negative in strain TA98 for mutations (Waskell 1978). Positive in *S. typhimurium* (TA100, 1535) for mutations (McCann et al. 1975). Positive in *S. typhimurium* (BA13, BAL13) for mutations (Roldán-Arjona et al. 1990). Positive in *S. typhimurium* (TM677) for mutations (Skopek and Thilly 1983). Positive in *S. typhimurium* (SV50) for mutations (Xu et al. 1984). Positive in *S. typhimurium* (TA1535/pSK1002) for mutations (Vericat et al. 1986). Positive in *S. typhimurium* (TA100) for mutations (Probst et al. 1981). Positive in *E. coli* (WP2, WP2 uvrA) for mutations (Probst et al. 1981). Positive in *E. coli* (K12) for mutations (Mohn and Van Zeeland 1985).
Epidemiological studies	A retrospective cohort study was conducted between 1950 and 1977 for the Exxon Corp, Baton Rouge ethanol and isopropanol plant. The study contained two study groups, the "process worker cohort" (335 workers) and the "total cohort" (743 workers, process workers + others) for which a standardized morbidity ratio (SMR) for laryngeal cancer was calculated, as a measure of relative risk. Based on the unit history and interviews of unit supervisors, exposure to diethyl sulfate, sulfuric acid mist, and other chemicals was routinely expected for the process workers. An SMR of 5.04 (95%CI 1.36–12.90) for the relative risk of laryngeal cancer was reported for the "process worker cohort" while an SMR of 3.2 (95%CI 1.3–6.6) was reported in the "total cohort." The investigators concluded that "it appears likely from the data that diethyl sulfate was the primary carcinogen." It was noted by the study authors, however, that their conclusion was complicated by the possible co-exposure to several other suspect carcinogens (Lynch et al. 1979). [Note that the 95% CI were not provided in the original article but were reported directly from IARC (1992). The IARC Working Group noted the co-exposure to sulfuric acid in the studied workers] A follow-up nested case-control study to Lynch et al. (1979) reported an association of upper respiratory tract cancers to high sulphuric acid exposures. Fifty histologically confirmed cases of primary upper respiratory tract cancer from all employees of the Exxon Baton Rouge plant employed for at least 12 months between 1944 and 1980 were matched with at least three controls each without respiratory cancer from the same population (n=175). Association of upper respiratory tract cancer and high sulfuric acid exposures vs. no/low sulfuric acid exposure were reported (n=50 cases unadjusted for confounding, OR=4.0, 95%CI 1.26–12.70). The investigators reported an association between sulfuric

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Endpoint	Lowest effect levels[ab]/Results
	acid exposures (high exposure vs. no/low exposure) and all upper respiratory tract cancer cases (n=43 cases adjusted for tobacco, alcohol and previous ENT disease, estimated OR=5.2, 95%CI 1.23–22.09). A strong association of sulphuric acid exposure with laryngeal cancer (n=30 cases adjusted for tobacco, alcohol, ENT disease, estimated OR=13.4, 95%CI 2.08–85.99) was also reported The positive association with high sulfuric acid exposure was still evident when the laryngeal cancer cases from the ethanol study by Lynch et al. (1979) were excluded (n=35 cases adjusted for confounding, OR=5.4, 95%CI 1.18–24.30) (Soskolne et al. 1984). [The IARC Working Group noted that this finding supports the role of sulfuric acid independent of dialkyl sulfates; however, it does not preclude a role for dialkyl sulfates (IARC 1992)] In a nested case-control study of the Union Carbide Texas City plastics plant, primary brain tumours (gliomas) were identified from previous retrospective cohort mortality studies of overall mortality experience (Waxweiler et al. 1983, Austin and Schnatter 1983) of employees from the Union Carbide Corporation (UCC) plastics plant in Texas City. 17 cases of deaths from malignant brain tumours (gliomas) in employees working at the UCC Texas City plant from 1950 to 1977 were matched to at least 6 non-brain cancer controls taken from the cohort of employees ever having worked at the plant. Investigators reported that "no significant differences between cases and controls were apparent in duration of exposure to any of the chemicals studied" (Leffingwell et al. 1983). In a case-control study of the Union Carbide Texas City plastics plant, cases of 21 primary brain tumours from former employees of the UCC Texas City plastics plant were reported. The 21 cases included 17 gliomas (as reported in Leffingwell et al. 1983, see above study) and 4 additional meningiomas. Cases were matched against two control groups of 80 males each selected from deceased employees of the UCC Texas City plant; one control group was a strictly non-cancer group. The investigators reported that no evidence of a significant association between exposures to specific chemicals and employment at the Texas City plant was found (Austin and Schnatter 1983). In a retrospective cohort study at the Union Carbide alcohol production plants in South Charleston and Texas City, a cohort of mostly Caucasian male workers (total n=1031) assigned to the production of ethanol and isopropanol at either the South Charleston plant (n=538 ever assigned to ethanol or isopropanol production from 1940 to 1978, strong-acid process only) or the Texas City plant (n=493 workers assigned for one month or more in the ethanol or isopropanol units, both strong- and weak-acid processes) were assessed. Statistical significant increases for lymphosarcoma and reticulosarcoma (5 cases vs. 0.9 expected, SMR=5.60, 95%CI 1.8–13.0) for the South Charleston plant was reported. However, this effect was not confirmed in the Texas City plant and was not further discussed by the investigators (Teta et al. 1992) [The IARC Working Group noted the lack of empirical exposure data for diethy sulfate in the above studies, the possibility of co-exposure to other potentially carcinogenic substances, and overall considered the epidemiology data as "*inadequate evidence* for the carcinogenicity in humans of diethyl sulfate" (IARC 1999).]

[a] LD_{50} = median lethal dose; LC_{50} = median lethal concentration; LOEL = lowest-observed-effect level.
[b] Conversion factor: $mg/m^3 = 6.31 \times ppm$ (IARC 1999).