DIETHYL SULFATE

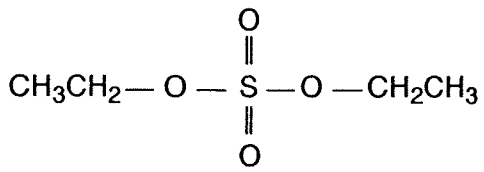
Diethyl sulfate was considered by previous IARC Working Groups, in 1973 and 1987 (IARC, 1974, 1987). Since then, new data have become available, and these are included in the present monograph and have been taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 64-67-5
Replaced CAS Reg. No.: 98503-29-8
Chem. Abstr. Name: Sulfuric acid, diethyl ester
IUPAC Systematic Name: Diethyl sulfate
Synonyms: Diethyl sulphate; diethyl tetraoxosulfate; DS; ethyl sulfate



$C_4H_{10}O_4S$ Mol. wt: 154.19

1.1.2 *Chemical and physical properties*

(*a*) *Description*: Colourless, oily liquid with faint peppermint odour (Sax & Lewis, 1987; Budavari, 1989)
(*b*) *Boiling-point*: 208–209.5 °C (decomposes) (Sax & Lewis, 1987; Budavari, 1989; Union Carbide Chemicals and Plastics Co., 1990)
(*c*) *Melting-point*: –25 °C (Budavari, 1989)
(*d*) *Density*: 1.1803 at 20 °C/20 °C (McCormack & Lawes, 1983; Sax & Lewis, 1987)
(*e*) *Spectroscopy data*: Infrared and nuclear magnetic resonance spectroscopy data have been reported (Aldrich Chemical Co., 1990).
(*f*) *Solubility*: Practically insoluble in water, 0.7 g/100 ml at 20 °C; miscible with ethanol and diethyl ether (McCormack & Lawes, 1983; Sax & Lewis, 1987; Budavari, 1989; Union Carbide Chemicals and Plastics Co., 1990)
(*g*) *Volatility*: Vapour pressure, 0.19 mm Hg [25 Pa] at 20 °C (Sax & Lewis, 1987); relative vapour density (air = 1), 5.31 (Budavari, 1989)
(*h*) *Stability*: Decomposes to diethyl ether, ethylene and sulfur oxides at temperatures above 100 °C (Union Carbide Chemicals and Plastics Co., 1990)

(*i*) *Reactivity*: Hydrolyses slowly in water (about 0.05%/h) at 25 °C to monoethyl sulfate and ethanol; reacts rapidly with water or aqueous alkali at temperatures above 50 °C; forms ethyl ether by reaction with ethanol. Diethyl sulfate is a strong alkylating agent (Budavari, 1989; Union Carbide Chemicals and Plastics Co., 1990).

(*j*) *Conversion factor*: $mg/m^3 = 6.31 \times ppm$[a]

1.1.3 *Technical products and impurities*

Diethyl sulfate is available as a technical-grade product with a minimal purity of 99.5% and a maximal acidity of 0.03% (calculated as sulfuric acid) (Union Carbide Chemicals and Plastics Co., 1989). It is also available as a laboratory chemical at a purity of $\geq$ 98% (American Tokyo Kasei, 1988; Aldrich Chemical Co., 1990; Janssen Chimica, 1990; Riedel-deHaen, 1990) or 95% (Eastman Fine Chemicals, 1990).

1.1.4 *Analysis*

An analytical method for the determination of diethyl sulfate in air involves adsorption of samples on silica gel, desorbtion with acetone and determination by gas chromatography using a flame photometric detector. The minimal concentration of diethyl sulfate detectable was 0.1 ppm [0.6 mg/m^3] (Gilland & Bright, 1980). A method for the analysis of diisopropyl sulfate in air (Kingsley *et al.*, 1984) can also be used for diethyl sulfate.

In a method for the analysis of diethyl sulfate in the work place, samples are adsorbed on a porous polymer such as Tenax TA, thermally desorbed and determined by gas chromatography using either flame ionization detection or flame photometric detection. The minimal quantities detectable were 0.1 ng with flame ionization detection and approximately 1 ng with flame photometry (Düblin & Thöne, 1988).

1.2 Production and use

1.2.1 *Production*

Commercial manufacture of diethyl sulfate starts with ethylene and 96 wt% sulfuric acid heated at 60 °C. The resulting mixture of 43 wt% diethyl sulfate, 45 wt% ethyl hydrogen sulfate and 12 wt% sulfuric acid is heated with anhydrous sodium sulfate under vacuum, and diethyl sulfate is obtained in 86% yield; the commercial product is > 99% pure. Dilution of the ethylene–sulfuric acid concentrate with water and extraction gives a 35% yield. In the reaction of ethylene with sulfuric acid, losses can occur due to several side reactions, including oxidation, hydrolysis–dehydration and polymerization, especially at sulfuric acid concentrations > 98 wt% (McCormack & Lawes, 1983).

Diethyl sulfate is believed to be produced commercially by two companies, one in the USA and one in Japan. Annual US production is estimated at 5000 tonnes.

Diethyl sulfate is an intermediate in the indirect hydration (strong acid) process for the production of ethanol involving ethylene and sulfuric acid. The reaction of ethylene with sulfuric acid is complex, and water plays a major role in determining the concentrations of the intermediate alkyl sulfates.

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[a]Calculated from: mg/m^3 = (molecular weight/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg [101.3 kPa])

$$CH_2=CH_2 + H_2SO_4 \rightarrow CH_3-CH_2-O-SO_3H$$

$$CH_3-CH_2-O-SO_3H + CH_2=CH_2 \rightarrow (CH_3-CH_2)_2SO_4$$

$$(CH_3-CH_2)_2SO_4 + H_2SO_4 \rightleftarrows 2CH_3-CH_2-O-SO_3H$$

$$(CH_3-CH_2)_2SO_4 + H_2O \rightarrow CH_3-CH_2-O-SO_3H + CH_3-CH_2OH$$

$$CH_3-CH_2-O-SO_3H + H_2O \rightarrow CH_3-CH_2OH + H_2SO_4$$

In ethanol production, the more water present in the extracting acid, the less ethylene is absorbed to produce the initial monoethyl sulfate. In addition, the more water that is present, the more monoethyl sulfate, once formed, is converted to ethanol. Diethyl sulfate can also be removed by rapid hydrolysis with acidic water. Therefore, increasing the water content in the sulfuric acid decreases the concentration of diethyl sulfate in the acid extract. Efficient ethanol production requires use of at least 90% sulfuric acid in the absorber. For example, in the Exxon Baton Rouge ethanol plant, 98.5% sulfuric acid is used (Lynch *et al.*, 1979). Details of this industrial process are given in the monograph on occupational exposure to mists and vapours from sulfuric acid and other strong inorganic acids (pp. 43–44).

1.2.2 *Use*

Diethyl sulfate is used chiefly as an ethylating agent in organic synthesis. The principal uses are as an intermediate in dye manufacture, as an ethylating agent in pigment production, as a finishing agent in textile manufacture and as a dye-set agent in carbonless paper. Smaller applications are in agricultural chemicals, in household products, in the pharmaceutical and cosmetic industries, as a laboratory reagent, as an accelerator in the sulfation of ethylene and in some sulfonation processes (McCormack & Lawes, 1983; Sax & Lewis, 1987; Budavari, 1989).

1.3 Occurrence

1.3.1 *Natural occurrence*

Diethyl sulfate is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data on occupational levels of exposure to diethyl sulfate were available to the Working Group.

On the basis of the US National Occupational Exposure Survey, the US National Institute for Occupational Safety and Health (1990) estimated that 2260 workers were potentially exposed to diethyl sulfate in the USA in 1981–83, in textile mills and the lumber and wood industries. Exposure to diethyl sulfate could also occur during its production or its use in the synthesis of a variety of intermediates and products (Center for Chemical Hazard Assessment, 1985). Exposure to diethyl sulfate in ethanol manufacturing plants has been inferred from its presence at concentrations ~30% in acid extracts. The maximal vapour concentration over a spill was calculated as 2000 ppm [12 620 mg/m^3]. Actual exposure of workers from spills or leaks would probably be much less, because of dilution in the surrounding air.

An analysis of historical records and interviews with unit supervisors in a US ethanol production plant indicated that there was frequent opportunity for exposure to diethyl

sulfate, since the equipment had to be opened often to clean sticky deposits in absorbers and extract soakers and since there was almost continual leakage from extract pump seals (Lynch *et al.*, 1979). Diethyl sulfate might be inhaled as aerosol during the opening of reaction vessels (Teta *et al.*, 1992).

Other potential exposures encountered in these processes are described in the monograph on occupational exposure to mists and vapours from sulfuric acid and other inorganic acids.

1.3.3 *Environmental occurrence*

In 1989, total air emissions of diethyl sulfate in the USA were estimated at approximately 4 tonnes from 28 locations; total land releases were estimated at 114 kg (US National Library of Medicine, 1991).

Diethyl sulfate has not been identified in the atmosphere. A study of the atmospheric chemistry of gaseous diethyl sulfate found no evidence for the formation of diethyl sulfate during the ozonolysis of olefins in the presence of sulfur dioxide and ethanol (Japar *et al.*, 1990).

1.4 Regulations and guidelines

The technical guiding concentration (TRK) of 0.2 mg/m^3 for diethyl sulfate, valid in Germany in 1985, was cancelled in 1989, and this compound was classified as III A2, compounds that 'have proven so far to be unmistakably carcinogenic in animal experimentation only; namely under conditions which are comparable to those for possible exposure of a human being at the workplace, or from which such comparability can be deduced' (Cook, 1987; Deutsche Forschungsgemeinschaft, 1989). No threshold limit value is applicable to diethyl sulfate, because it is considered to be carcinogenic in several countries (e.g., Finland, France, Sweden) (International Labour Office, 1991).

2. Studies of Cancer in Humans

2.1 Cohort studies

A historical cohort study was conducted of 335 US workers in ethanol and isopropanol units (Lynch *et al.*, 1979), described in detail on p. 81. The relative risk for developing laryngeal cancer was 5.04 [95% confidence interval (CI), 1.36–12.90], based on four cases. When the cohort was expanded to include mechanical craftsmen and supervisors, for a total of 740 men, the relative risk was 3.2 [95% CI, 1.3–6.6] based on seven cases. The ethanol process involved strong concentrations of sulfuric acid (98.5% wt), while the isopropanol process involved sulfuric acid at 'weak' concentrations (65–75 wt%). Dialkyl sulfates are generated as intermediates in these processes, but the ethanol process generated some 30 times more than the weak-acid process, owing to the use of more concentrated acid. The excess risk was determined for the two process units combined and was tentatively attributed to the dialkyl (diethyl and diisopropyl) sulfates. [The Working Group noted that the excess risk was determined over two units in which sulfuric acid was used at different concentrations.]

The mortality experience of 1031 ethanol and isopropanol process workers in two plants in the USA (Teta *et al.*, 1992) was determined as an extension to the study by Weil *et al.* (1952) (described on pp. 80–82). The mortality patterns of the combined cohort of strong-acid workers were markedly different from those of weak-acid workers, among whom no cancer death was seen. In the strong-acid group, two laryngeal cancers and three buccal cavity and pharyngeal cancers were observed, giving elevated but nonsignificant standardized mortality ratios; mortality from lung cancer was not increased. The authors recognized the lack of power in their study to detect significant effects.

2.2 Case–control studies

A nested case-control study comprising 17 glioma deaths and six controls each was conducted among workers in a US petrochemical plant in 1950–77 (Leffingwell *et al.*, 1983). Controls without cancer were individually matched on race, sex and year of birth (within three years); the year of first employment for each control was not earlier than three years before that of the case; the date of last employment was later for controls than for the case. Possible associations between gliomas of the brain and job title, employment history by department, history of chemical exposure, location within the plant, dates of employment and residence were examined. Estimated exposure to diethyl sulfate gave an odds ratio of 2.10 (90% CI, 0.57–7.73); duration of exposure was not related to disease status. In a parallel analysis of 21 brain tumours [including the 17 gliomas studied by Leffingwell *et al.* (1983)], which used a different series of controls, the proportion of cases exposed was similar to that of controls (Austin & Schnatter, 1983).

Soskolne *et al.* (1984) conducted a case–control study (described in detail on p. 89) to examine the role of exposure to sulfuric acid in laryngeal cancer at the same plant studied by Lynch *et al.* (1979). They found a high correlation with exposure to sulfuric acid in any of three processes (strong-, intermediate- or weak-acid). Exposure–response relationships were seen. Similar results were obtained even after exclusion of those cases studied by Lynch *et al.* (1979) that were associated with the ethanol and isopropanol units. [The 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.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Two groups of 12 BD rats [sex unspecified], about 100 days old, received 25 or 50 mg/kg bw diethyl sulfate [purity unspecified] in arachis oil once weekly by gavage for 81 weeks (total dose, 1.9 or 3.7 g/kg bw) and were observed until death [time of death unspecified]. One squamous-cell carcinoma of the forestomach was found in each group, and 6/24 rats [distribution of tumours by group unspecified] had a number of benign papillomas of the forestomach (Druckrey *et al.*, 1970). [The Working Group noted the small number of animals used and the absence of a concurrent control group.]

3.2 Subcutaneous administration

Rat: Two groups of 12 BD rats [sex unspecified], about 100 days old, received subcutaneous injections of 25 or 50 mg/kg bw diethyl sulfate [purity unspecified] in arachis oil (concentrations, 1.25 or 2.50%) once weekly for 49 weeks (total dose, 0.8 or 1.6 g/kg bw) and were observed until death (295–685 days). Local tumours (three spindle-cell sarcomas, three fibrosarcomas, three myosarcomas, one polymorphocellular sarcoma and one glandular carcinoma of unknown origin) developed at the site of injection in the 11 surviving rats in the high-dose group during a mean survival time of 350 ± 50 (standard deviation) days (one rat in this group died prematurely from pneumonia). Two cases of metastasis to the lungs occurred. Local tumours (three fibrosarcomas, two spindle-cell sarcomas, one myosarcoma) developed at the site of injection in 6/12 rats in the low-dose group, during an average survival period of 415 days [standard deviation unspecified] (Druckrey *et al.*, 1970). [The Working Group noted the absence of a concurrent control group and that historical vehicle controls had no local tumour even when injected subcutaneously with high doses of the vehicle.]

3.3 Other experimental systems

Rat: A single subcutaneous injection of 85 mg/kg bw (25% of LD_{50}) diethyl sulfate [purity unspecified; vehicle most probably arachis oil] was given to three pregnant BD rats [age unspecified] on day 15 of gestation. One of the rats died with multiple mammary gland carcinomas at the age of 742 days. Thirty offspring [sex unspecified] were observed until death; two developed malignant neurinomas, one of the cauda equina (in a rat found dead at the age of 285 days) and one of the lumbal nerve (in a rat dead at day 541). Spontaneous tumours of this type had not been observed in untreated historical control BD rats (Druckrey *et al.*, 1970). [The Working Group noted the small number of pregnant females treated and the absence of a concurrent control group.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group

4.1.2 *Experimental systems*

After male CFE albino rats were administered 1 ml of a 5% (v/v) solution of diethyl sulfate in arachis oil by gavage or by intraperitoneal or subcutaneous injection, ethylmercapturic acid and a sulfoxide were identified as metabolites (Kaye, 1974).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The LD_{50}s of diethyl sulfate have been summarized as 150 mg/kg bw by intraperitoneal injection in mice, 350 mg/kg bw by subcutaneous injection in rats, 350–1000 mg/kg bw by oral administration in rats, and 600 mg/kg bw by percutaneous administration in rabbits (Druckrey *et al.*, 1970; Deutsche Forschungsgemeinschaft, 1990).

Diethyl sulfate is a strong skin irritant (Sax & Lewis, 1987; Deutsche Forschungsgemeinschaft, 1990).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a mammalian spot test, C57 × T-stock mice were treated intraperitoneally with 0–225 mg/kg bw diethyl sulfate on day 10.25 of gestation. No effect on litter size at birth was noted (Braun *et al.*, 1984).

4.4 Genetic and related effects (see also Table 1 and Appendices 1 and 2)

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

The genetic effects of diethyl sulfate have been reviewed (Hoffmann, 1980). It reacts with DNA *in vitro* to produce, primarily, ethylation at the *N*7 position of guanine (Lawley, 1966). It induced SOS repair and forward and reverse mutations in bacteria and mitotic recombination and mutation in *Saccharomyces cerevisiae*.

Diethyl sulfate induced unscheduled DNA synthesis in pollen of *Petunia hybrida*. It induced chromosomal aberrations in meiotic cells and chlorophyll mutations in rice, ring chromosomes in *Allium sativum* root tips and anaphase and telophase aberrations in *Papaver somniferum* root meristemic cells.

Sex-linked recessive lethal mutations were induced in *Drosophila melanogaster* after larval feeding and after exposure to diethyl sulfate vapour. Larval feeding of this compound induced crossing-over and autosomal recessive lethal mutations, but it did not induce reciprocal translocations between the second and third chromosomes in male *D. melanogaster*.

Diethyl sulfate induced DNA single-strand breaks in Chinese hamster ovary (CHO) cells and unscheduled DNA synthesis in primary cultures of adult rat hepatocytes. Mutations were induced at the *hprt* and Na^+/K^+ ATPase loci in CHO cells and Chinese hamster lung (V79) cells. Diethyl sulfate induced sister chromatid exchange in V79 cells. It induced alkali-labile sites, numerical and structural aberrations and micronuclei in cultured human lymphocytes.

Micronucleated erythrocytes were induced in larvae of newts (*Pleurodeles waltl*) treated with diethyl sulfate. In mice, diethyl sulfate alkylated DNA to produce mainly

Table 1. Genetic and related effects of diethyl sulfate

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS functions, *Escherichia coli*	+	0	40.0000	Barbé *et al.* (1983)
PRB, SOS functions, *Escherichia coli*	+	0	1170.0000	Vericat *et al.* (1986)
PRB, SOS functions, *Escherichia coli*	+	0	30.0000[c]	de Oliveira *et al.* (1986)
PRB, *umu* test, *Salmonella typhimurium* TA1535/pSK1002	0	+	0.2000	Nakamura *et al.* (1987)
SAF, *Salmonella typhimurium* SV50 (Ara^r), forward mutation	+	0	75.0000	Xu *et al.* (1984)
SAF, *Salmonella typhimurium* BA13 (Ara^r), forward mutation	+	0	154.0000	Roldán-Arjona *et al.* (1990)
SAF, *Salmonella typhimurium* TM677, forward mutation	+	0	65.0000	Skopek & Thilly (1983)
ECK, *Escherichia coli* K12-343/113, forward mutation	+	0	308.0000	Mohn & van Zeeland (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	0.0000	McCann *et al.* (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2500.0000[c]	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0[d]	0.0000	Probst *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	0.0000	McCann *et al.* (1975)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	2500.0000[c]	Waskell (1978)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	0	5000.0000[c]	Levin *et al.* (1982)
SAS, *Salmonella typhimurium* TS1121, reverse mutation	+	0	600.0000	Hoffmann *et al.* (1988)
SAS, *Salmonella typhimurium* TS1157, reverse mutation	+	0	600.0000	Hoffmann *et al.* (1988)
SAS, *Salmonella typhimurium* TA90, reverse mutation	+	0	5000.0000	Levin *et al.* (1982)
SAS, *Salmonella typhimurium* TA2637, reverse mutation	-	0	5000.0000	Levin *et al.* (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	0	2500.0000	Levin *et al.* (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	0	2500.0000	Levin *et al.* (1982)
SAS, *Salmonella typhimurium* TA88, reverse mutation	+	0	2500.0000	Levin *et al.* (1982)
SAS, *Salmonella typhimurium* TR3243 (*his* D6610), reverse mutation	+	0	5000.0000	Levin *et al.* (1982)
SAS, *Salmonella typhimurium his*C3076, reverse mutation	+	0	2500.0000	Levin *et al.* (1982)
SAS, *Salmonella typhimurium his*D3052, reverse mutation	-	0	2500.0000	Levin *et al.* (1982)
ECW, *Escherichia coli* WP2 *uvr*A$^-$, mutation	+	0[d]	0.0000	Probst *et al.* (1981)
EC2, *Escherichia coli* WP2, mutation	+	0[d]	0.0000	Probst *et al.* (1981)
SCH, *Saccharomyces cerevisiae* D1, mitotic recombination	+	0	4500.0000[c]	Zimmermann *et al.* (1966)

Table 1 (contd)

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCR, *Saccharomyces cerevisiae* D1, reverse mutation	+	0	4500.0000[c]	Zimmermann *et al.* (1966)
PLU, *Petunia hybrida*, Mature pollen, unscheduled DNA synthesis	+	0	15400.0000	Jackson & Linskens (1980)
ACC, *Allium cepa* root-tip cells, chromosomal aberrations	-	0	4600.0000	Gohil & Kaul (1983)
PLC, *Papaver somniferum*, chromosomal aberrations	+	0	1200.0000	Floria & Ghiorghita (1980)
PLC, *Allium sativum* root-tip cells, chromosomal aberrations	+	0	3850.0000	Gohil & Kaul (1983)
PLM, *Oryza sativa* (rice) chlorophyll mutations	+	0	600.0000	Reddy *et al.* (1974)
PLC, *Oryza sativa* (rice), chromosomal aberrations	+	0	350.0000	Seetharami Reddi & Reddi (1985)
DMG, *Drosophila melanogaster*, genetic crossing-over	+	0	6000.0000[c]	Pelecanos (1966)
DMM, *Drosophila melanogaster*, autosomal recessive lethal mutation	+	0	6000.0000[c]	Pelecanos (1966)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	0.0000	Abraham *et al* (1979)
DMX, *Drosophila melanogaster mei*-9^{LI}, sex-linked recessive lethal mutation	+	0	1500.0000[c]	Vogel (1989)
DMX, *Drosophila melanogaster*, exr^{+}, sex-linked recessive lethal mutation	+	0	1500.0000[c]	Vogel (1989)
**Drosophila melanogaster*, sex chromosome loss	-	0	0.0000 (vapour, 5–15 min)	Abraham *et al.* (1979)
DMC, *Drosophila melanogaster*, reciprocal translocation 2nd–3rd chromosome	-	0	6000.0000	Pelecanos (1966)
DIA, DNA single-strand breaks, Chinese hamster ovary cells *in vitro*	+	0	154.0000	Abbondandolo *et al.* (1982)
DIA, DNA single-strand breaks, Chinese hamster ovary cells *in vitro*	+	0	385.0000[c]	Dogliotti *et al.* (1984)
URP, Unscheduled DNA synthesis, rat primary hepatocyte cells	+	0	15.4000	Probst *et al.* (1981)
GCO, Gene mutation ($6TG^{r}$), Chinese hamster ovary K1-BH4 cells	+	0	46.0000	Couch *et al.* (1978)
GCO, Gene mutation ($6TG^{r}$), Chinese hamster ovary K1 cells	+	0	154.0000	Bignami *et al.* (1988)
GCO, Gene mutation (Oua^{r}), Chinese hamster ovary K1 cells	+	0	154.0000	Bignami *et al.* (1988)
G9H, Gene mutation ($6TG^{r}$), Chinese hamster V79 cells *in vitro*	+	0	100.0000	Nishi *et al.* (1984)
G9H, Gene mutation ($6TG^{r}$), Chinese hamster V79 cells *in vitro*	+	0	308.0000	Mohn & van Zeeland (1985)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	0	100.0000	Nishi *et al.* (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIA, Micronucleus test, Chinese hamster V79 cells *in vitro*	+	0	154.0000	Bonatti *et al.* (1986)
MIA, Micronucleus test, Chinese hamster V79 cells *in vitro*	+	0	40.0000	De Ferrari *et al.* (1988)
MIA, Micronucleus test, Chinese hamster V79 cells *in vitro*	+	0	460.0000	Nüsse *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	0	100.0000	Asita (1989)
DIH, Alkali-labile site, human leukocytes *in vitro*	+	0	154.0000	Schutte *et al.* (1988)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	0	154.0000	De Ferrari *et al.* (1988)
AIH, Chromosomal aberrations (numerical), human lymphocytes *in vitro*	+	0	15.0000	De Ferrari *et al.* (1988)
CHL, Chromosomal aberrations (structural), human lymphocytes *in vitro*	+	0	154.0000	De Ferrari *et al.* (1988)
DVA, Alkaline elution, Sprague-Dawley rat brain cells *in vivo*	+	0	40.0000[c]	Robbiano & Brambilla (1987)
BVD, DNA adduct formation, mouse germ/testis/bone-marrow/liver *in vivo*	+	0	48.0000	van Zeeland *et al.* (1990)
MST, Mouse spot test, C57Bl/6 Jena × T stock *in vivo*	?	0	225.0000	Braun *et al.* (1984)
SLP, Mouse specific locus test *in vivo*	(+)	0	200.0000	Ehling & Neuhäuser-Klaus (1988)
MVM, Micronucleus test, ddY mouse peripheral blood cells *in vivo*	+	0	400.0000	Asita *et al.* (1992)
*Micronucleus test, *Pleurodeles waltl* larvae erythrocytes	+	0	6.0000 in water	Jaylet *et al.* (1986)
COE, Chromosomal aberrations, embryonic NMRI mouse cells *in vivo*	+	0	150.0000	Braun *et al.* (1986)
DLM, Dominant lethal test, mice *in vivo*	+	0	100.0000	Ehling & Neuhäuser-Klaus (1988)

[a] +, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)
[b] In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[c] Single dose level tested
[d] Result not clear
*Not displayed on profile

*N*7-ethylguanine in germ cells, testis tubuli, bone marrow and liver (van Zeeland *et al.*, 1990). Brain DNA was fragmented in male rats treated intraperitoneally with diethyl sulfate. An inconclusive result was obtained in the mouse somatic coat colour mutation test (spot test). Diethyl sulfate induced specific locus mutations in mouse germ-line cells at 200 mg/kg but not at 300 mg/kg. It induced dominant lethal mutations and chromosomal aberrations, which were mainly chromatid breaks and gaps, in mouse embryonal cells after transplacental treatment. It induced micronuclei in mouse peripheral reticulocytes.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Diethyl sulfate is manufactured from ethylene and sulfuric acid. It is used principally as an intermediate (ethylating agent) in the manufacture of dyes, pigments and textile chemicals, and as a finishing agent in textile production. It is an obligatory intermediate in the indirect hydration (strong acid) process for the preparation of synthetic ethanol from ethylene.

No data were available on levels of occupational exposure to diethyl sulfate.

5.2 Human carcinogenicity data

One cohort study at a US isopropanol and ethanol manufacturing plant revealed an increased risk for laryngeal cancer. A subsequent case–control study nested in an expanded cohort at this plant indicated that the increased risk was related to exposure to sulfuric acid; the risk persisted even after exclusion of workers in the ethanol and isopropanol units. A cohort study from two US plants producing ethanol and isopropanol suggested an increased risk for cancers of the larynx, buccal cavity and pharynx, but not of the lung, in strong-acid workers. An association between estimated exposure to diethyl sulfate and risk for brain tumour was suggested in a study of workers at a US petrochemical plant.

No measurement of exposure diethyl sulfate was available for the industrial processes investigated in the epidemiological studies. It is therefore difficult to assess the contribution of diethyl sulfate to the increased cancer risks. Furthermore, exposure to mists and vapours from strong inorganic acids, primarily sulfuric acid, may play a role in increasing these risks.

5.3 Animal carcinogenicity data

Diethyl sulfate was tested for carcinogenicity by oral and subcutaneous administration in one strain of rats. After subcutaneous administration, a high incidence of malignant tumours occurred at the injection site. Following oral gavage of diethyl sulfate, forestomach tumours were observed. A low incidence of malignant tumours of the nervous system was observed in the same strain of rats after prenatal exposure.

5.4 Other relevant data

Diethyl sulfate induced specific locus mutations in mouse germ-line cells. It was clastogenic in mice and newts, induced DNA damage in mice and rats and ethylated DNA in

mice. Diethyl sulfate induced chromosomal aberrations and micronucleus formation in cultured human lymphocytes. It induced alkali-labile sites in cultured human leukocytes in one study. In cultured mammalian cells, diethyl sulfate induced chromosomal aberrations, micronucleus formation, sister chromatid exchange, forward mutation and DNA single-strand breaks; it also induced unscheduled DNA synthesis in primary cultures of rat hepatocytes. In single studies, diethyl sulfate did not induce aneuploidy or reciprocal translocation in *Drosophila melanogaster* but did induce sex-linked recessive lethal mutations and genetic crossing-over. In plant cells, diethyl sulfate induced chromosomal aberrations, mutation and unscheduled DNA synthesis. It induced reverse mutation and mitotic recombination in yeast. Diethyl sulfate induced mutation and DNA damage in bacteria.

5.5 Evaluation[1]

There is *inadequate evidence* for the carcinogenicity in humans of diethyl sulfate.

There is *sufficient evidence* for the carcinogenicity in experimental animals of diethyl sulfate.

Diethyl sulfate is a strong alkylating agents which ethylates DNA. As a result, it is genotoxic in virtually all test systems examined including induction of potent effects in somatic and germ cells of mammals exposed *in vivo*.

Overall evaluation

Diethyl sulfate *is probably carcinogenic to humans (Group 2A).*

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[1]For definition of the italicized terms, see Preamble, pp. 26–29.

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