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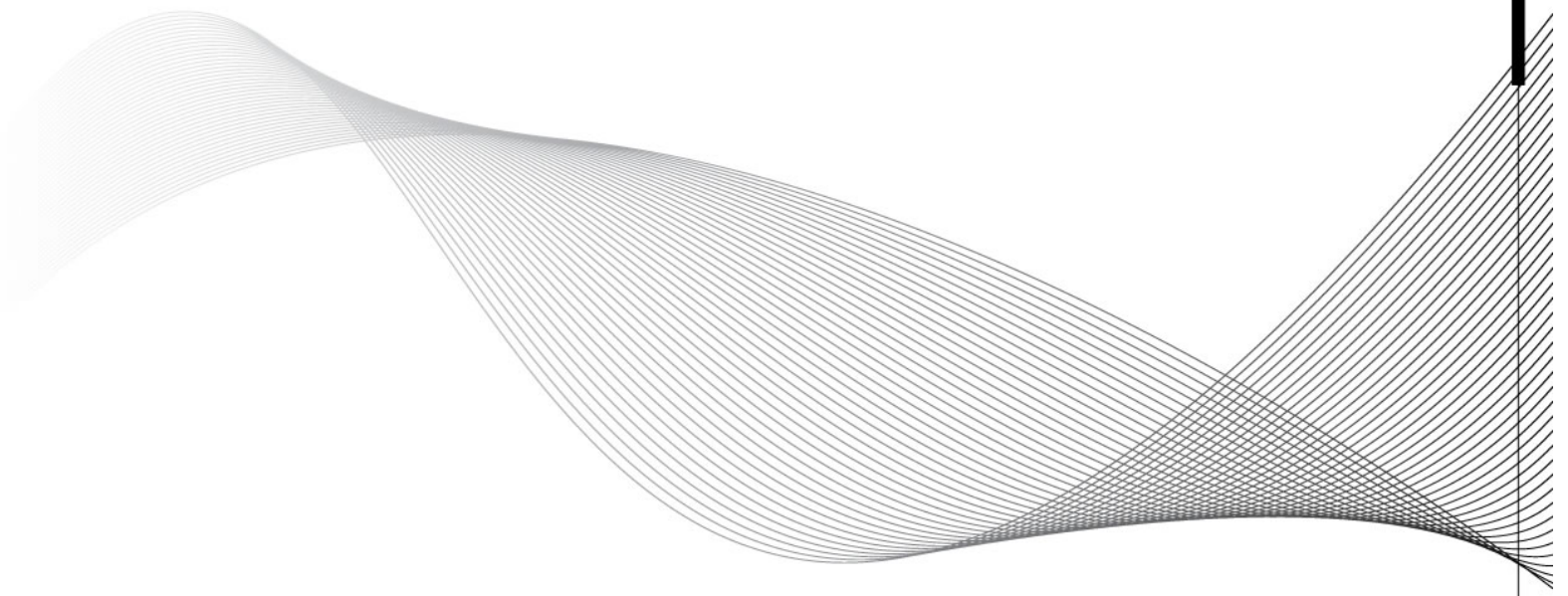

Recommendation from the Scientific Committee on Occupational Exposure Limits for diethyl sulphate

SCOEL/SUM/154

December 2009

European Commission

Table of Contents

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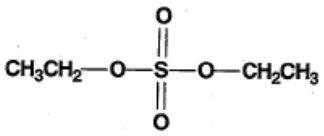
8 hour TWA:	not applicable (see Recommendation)
STEL (15 min):	not applicable (see Recommendation)
Additional classification:	S (skin absorption)
SCOEL carcinogen group:	A (non-threshold carcinogen)

Substance: Diethyl sulphate



Synonyms: ethyl sulphate, sulphuric acid diethyl ester, diethyl monosulphate

EINECS No: 016-027-00-6

EC No: 200-589-6

Classification: Carcinogenicity Cat. 2; R45, Mutagenicity Cat. 2, R46, Xn; R20/21/22, C; R34

CAS No: 64-67-5

MWt: 154.19 g/mol

Conversion factor (20°C, 101 kPa): 6.409 mg/m^3 = 1 ppm; 0.1156 ppm = 1 mg/m^3

This document is based on the MAK documentations from 1979 and 2000 (DFG, 1980, 2000) and the IARC documentations (IARC 1992, 1999). This was supplemented by a literature survey of SCOEL and by results of recent discussions in Germany by AGS/UAIII.

Physico-chemical properties:

Dimethyl sulphate is a colour- and odourless oily liquid with a melting point of about -25°C and a boiling point of 209.5 °C (decomposition). It is not flammable and not explosive.

1. Occurrence/use

Diethyl sulphate is used principally as an ethylating agent in the manufacture of dyes, pigments and textile chemicals, and as a finishing agent in textile production. It is an intermediate in the "strong acid process" for the preparation of synthetic ethanol from ethylene. No data are available on levels of occupational exposure to diethyl sulphate (IARC, 1992).

2. Health significance

2.1. Toxicokinetics/metabolism

The metabolism of diethyl sulphate has been studied in rats (s.c., i.p., oral administration; Kaye 1974). The metabolism proceeds *via* conjugation to glutathione; the urinary excretion product is ethyl mercapturic acid. Owing to its alkylating properties diethyl sulphate reacts with DNA, primarily at N7 of guanine, and to minor extents at several positions of adenine and cytosine (DFG 1980).
Based on the structural similarity to dimethyl sulphate (SCOEL/SUM/111) a skin absorption of diethyl sulphate appears likely, although experimental or human data are not available.

2.2. Acute toxicity

The acute toxicity of diethyl sulphate is somewhat less that that of dimethyl sulphate. LD-50 values in different species (rats, mice, rabbits; oral, s.c., i.p.) vary between 150 and 880 mg/kg. Inhalation exposures of rats, for 4 h to 500 ppm, were lethal (DFG 1980). The clinical picture of acute poisoning by inhalation is similar to that of other alkylating agents; animals die from lung oedema after 10-20 hours (DFG 1980).
There are no human data on acute toxicity.

2.3. Irritation and corrosivity

According to older publications diethyl sulphate is clearly caustic to the skin, although to a lesser extent that dimethyl sulphate (compiled by DFG 1980).

2.4. Repeated dose studies

See chapter on carcinogenicity (*v.i.*).

2.5. Mutagenicity

There are no published data in humans.

As summarized by IARC (1992, 1999), diethyl sulphate induced mutation and DNA damage in bacteria and induced reverse mutation and mitotic recombination in yeast. In the Ames test with *S. typhimurium* it is directly mutagenic and does not require metabolic activation (McCann 1975). In plant cells, diethyl sulphate induced chromosomal aberrations. In a single study, diethyl sulphate did not induce heritable translocation in *Drosophila melanogaster* but did induce autosomal recessive lethal mutations, sex-linked recessive lethal mutations and genetic crossing over. In cultured mammalian cells, diethyl sulphate induced chromosomal aberrations, micronucleus formation, sister chromatid exchanges, forward mutation and DNA single-strand breaks; it also induced unscheduled DNA synthesis in primary cultures of rat hepatocytes. Diethyl sulphate

induced chromosomal aberrations, micronucleus formation and aneuploidy in cultured human lymphocytes. It induced alkali-labile sites in cultured human leukocytes in one study. It was clastogenic in mice and newts *(Pleurodeles waltl),* induced DNA damage in mice and rats and ethylated DNA in mice. Diethyl sulphate induced specific locus mutations in mouse germ-line cells. In mice, diethyl sulphate alkylated DNA to produce mainly N7-ethylguanine in germ cells, testis tubules, bone marrow and liver (IARC, 1992).
DNA base sequence changes were analysed in 31 transmissible *vermilion* mutants recovered from *Drosophila melanogaster,* the male germ cells of which had been treated with diethyl sulphate. There were 93% base-pair substitutions and 7% deletions. The most frequent base-pair changes were GC-AT transitions (75%) and AT-TA transversions (10%) (Sierra *et al.,* 1993).

2.6. Carcinogenicity

2.6.1. Studies in humans

Industrial exposure to diethyl sulphate occurs primarily in ethanol production ("strong-acid process"). One cohort study at an isopropanol and ethanol manufacturing plant in the United States revealed a significantly increased risk for laryngeal cancer (standardized mortality ratio [SMR], 5.0 (95% CI, 1.4-12.9), based on four cases; after including some additional groups of workers, the SMR was 3.2 (95% CI, 1.3-6.6) based on seven cases (IARC, 1992).
A cohort study at two plants producing ethanol and isopropanol in the United States showed non-significant excess risks based on two cancers of the larynx and three buccal cavity and pharynx cancers in strong-acid workers (IARC, 1992). A subsequent case-control study nested in an expanded cohort at the aforementioned isopropanol and ethanol manufacturing plant in the United States indicated that the increased risk of laryngeal cancer was related to exposure to sulphuric acid; the risk persisted even after exclusion of workers in the ethanol and isopropanol units (IARC, 1992).
An association between estimated exposure to diethyl sulphate and risk for brain tumours was suggested in a case-control study of workers at a petrochemical plant in the United States. Seventeen glioma cases and six times as many controls were included and an odds ratio of 2.1 (90% confidence interval [CI], 0.6-7.7) was obtained; a parallel study of 21 cases (including the 17 of this other study) and with another set of controls showed no clear increase in risk (IARC, 1992).

2.6.2. Experimental studies in animals

No chronic inhalation studies have been reported. Long-term feeding and injection studies were performed with rats (see *Table 1*), with the following results:
After oral application (gavage) of 25 or 50 mg/kg BD rats (12 per group), once weekly, over 81 weeks and surveillance of the animals until their natural death there was 1 squamous cell carcinoma of the forestomach in each animal of the treated groups, and in 6 rats there was a (not reported) number of benign papillomas. The authors discussed that diethyl sulphate was quickly hydrolysed in the stomach and had therefore no systemic carcinogenic effect (Druckrey et al. 1970).
After weekly subcutaneous injections of 25 or 50 mg/kg to rats (12 per group) for 49 weeks all animals of the higher dose group developed local tumours at the injection site; one animal died from pneumonia. The tumours were sarcomas (spindle-cell, fibrosarcomas, myosarcomas, polymorph-cell sarcomas), and one adenocarcinoma. Six of the 12 rats of the lower dose group contracted local fibrosarcomas (3), spindle-cell sarcomas (2), or myosarcoma (1) (Druckrey et al. 1970).

After a single sub-cutaneous dose of 50 mg/kg diethyl sulphate to BD rats 17 out of 24 animals developed local tumours. The observation time was not noted, nor was there mentioning of control group(s).
The transplacental carcinogenicity of diethyl sulphate was also studied in preliminary form by Druckrey et al. (1970). Three pregnant rats received 85 mg/kg i.v. (about 1/4 LD-50) at the 15th gestational day. Thirty offspring animals were raised until their natural death. Malformations were not found, but 2 animals developed malignant tumours of the CNS (neurinomas). The mother animals later developed multiple mammary carcinomas (also reported in: Druckrey 1973).

Table 1: Experimental data regarding carcinogenic effects of diethyl sulphate; data by Druckrey et al. (1970) and Preussmann (1968), as compiled by DFG, 1980)

Animals,	application/solvent	dose (mg/kg)	duration	results/symptoms
BD rats, 12	s.c., 1.2% in oil	25 (total 0.8 g/kg)	1/week, 49 weeks	6 rats with local tumours
BD rats, 12	s.c., 2.5% in oil	59 (total 1.6 g/kg)	1/week 49 weeks	11 rats with local tumours
BD rats, 12	gavage, in oil	25 (total 1.9 g/kg)	1/week 81 weeks	1 forestomach ca. (sev. papillomas)
BD rats, 12	gavage, in oil	50 (total 3.7 g/kg)	1/week 81 weeks	1 forestomach ca. (sev. papillomas)
BD rats, 24	s.c., in oil	50	single dose	17 animals with local tumours

Although there are only older publications that do not match modern experimental standards, a strong carcinogenic activity of diethyl sulphate is evident (IARC 1974, 1992; DFG 1980, 2000).
Similar results had been obtained by the same group with the analogous compound, dimethyl sulphate (Druckrey et al. 1966, 1970; see DFG 1985). Hence, diethyl and dimethyl sulphate appear comparable regarding both their mode of carcinogenic action and their carcinogenic activity.
Diethyl sulphate was evaluated by IARC (1999) as "probably carcinogenic to humans (Group 2A)". This overall evaluation took also into account that diethyl sulphate 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 induction of potent effects in somatic and germ cells of mammals exposed *in vivo*.

2.7. Reproductive toxicity studies

Groups of adult female (C3H/R1 x 101/R1)F_1 mice were treated with diethyl sulphate by a single intraperitoneal injection of 150 mg/kg bw within four days before mating or at 1, 6, 9 or 25 h after mating with untreated males. Control groups were treated with vehicle only (0.1 mL dimethyl sulfoxide) four days before mating or 6 or 25 h after mating. Control and treated females were killed and their uterine contents examined 17-18 days after mating. Resorptions were significantly increased ($p < 0.01$) following treatment 1, 6 or 9 h after mating (30%, 24% and 14%, respectively) in comparison with available control group frequencies of 4.1%, 10% and 3.9%. Treatment had no effect if given before mating or 25 h

after mating. Midgestational and late deaths were significantly increased at 1 h (15% and 14%, respectively) and at 6 h (16% and 21%, respectively), in comparison with available control frequencies of 0.9% and 1.3%. No effect was observed at other times. The incidences of live fetuses with malformations were (numbers of fetuses examined in parentheses): before mating control, 0.6% (338), treated, 0.2% (441); 1 h after mating control, 0.3% (371), treated, 15% (113); 6 h treated, 25% (157); 9 h treated, 3% (213); 25 h treated, 2% (314). In contrast to other alkylating agents with similar DNA-binding properties but different effects upon exposed zygotes, there appeared to be no site-specific alkylation product identifiable as the critical target. The authors speculated that the lethal effects were due to an epigenetic disruption of gene expression during early embryogenesis (Generoso *et al.,* 1991; see IARC 1999).

Recommendation

Diethyl sulphate is a strong alkylating agent. It is clearly directly mutagenic in virtually all test systems examined. It has been tested for carcinogenicity by oral and subcutaneous administration in one strain of rats. After subcutaneous administration, a high incidence of malignant tumours at the injection site was observed. Following oral gavage of diethyl sulphate, tumours of the forestomach were observed. A low incidence of malignant tumours of the nervous system was observed in the same strain of rats after prenatal exposure (IARC, 1992). Hence, the compound is carcinogenic locally and systemically.

Inhalation carcinogenicity studies have not been performed. However, the available carcinogenicity data are generally comparable to those obtained with the structural analogue, dimethyl sulphate, for which long-term inhalation exposure conditions for rats at 0.5 ppm (6 h, twice per week) were clearly carcinogenic (see SCOEL/SUM/111). Hence, it is recommended that dimethyl sulphate and diethyl sulphate should be regulated and controlled in a similar way. Based on its very clearcut genotoxic and alkylating potency, the compound is assigned to the SCOEL carcinogen group A (non-threshold carcinogen; Bolt and Huici-Montagud 2008). A health-based OEL cannot be derived.

As for dimethyl sulphate, occupational exposures to diethyl sulphate should strictly be minimised, taking every possible technical precaution.

By analogy to dimethyl sulphate, a "skin notation" is applied.

No data are reported on biological monitoring of diethyl sulphate. By analogy to dimethyl sulphate (see SCOEL/SUM/111) it appears plausible that the haemoglobin adduct (N-ethylvaline at the N-terminus of haemoglobin) could be a useful parameter, which should be employed in occupation al field studies.

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