FOREWORD **INTRODUCTION**

TETRAHYDROTHIOPHENE-1,1-DIOXIDE

CAS N°: 126-33-0

SIDS Initial Assessment Report

For

SIAM 19

Berlin, Germany, 19–22 October 2004

1. Chemical Name:	Tetrahydrothiophene-1,1-dioxide
2. CAS Number:	126-33-0
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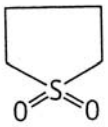
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	Sumitomo Seika Chemicals Co. Ltd. No consortium has been formed. Chevron Phillips have provided information, comments and recommendations to Sumitomo Seika.
• Process used	Industry collected data, prepared the updated IUCLID dossier, and drafted versions of the SIAR and SIAP.
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals	The substance is sponsored by Japan under the ICCA Initiative, and is submitted for first discussion at SIAM 19.

Programme?

7. Review Process Prior to the SIAM:	Japanese government peer-reviewed the documents and audited selected studies.
8. Quality check process:	Japanese government peer-review committee performed spot checks on randomly selected endpoints and compared original studies with data in the SIDS dossier
9. Date of Submission:	23 July 2004
10. Date of last Update:	14 January 2005
11. Comments:	None

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	126-33-0
Chemical Name	Tetrahydrothiophene 1,1-dioxide
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

In rats dosed intravenously at 500 mg/kg, 28%, 36% and 37% of the dose was excreted unchanged between days 0-2, 0-4 and 0-7, respectively. At 1000 mg/kg, 50% and 67.2% of the dose was excreted unchanged between days 0-2 and 0-4, respectively. The observation that the proportion of the dose recovered increased with dosage suggests that the metabolic pathway is saturable. In rabbits, dogs and squirrel monkeys given a single iv injection, this chemical was rapidly distributed throughout the body and was slowly removed from plasma with a half-life of 3.5-5 hours. One major metabolite with 85% of the urinary radioactivity was found in male rats injected intraperitoneally with this chemical. In a follow up study, the metabolite was identified as 3-hydroxysulfolane in the urine of rabbits injected intraperitoneally with this chemical.

LD_{50} values by gavage [OECD TG 401] were 2006 mg/kg (males) and 2130 mg/kg (females) in rats. Dermal LD_{50} in male and female rats was greater than 2000 mg/kg [84/449/EEC, B3]. Inhalation LC_{50} in male and female rats(four hours) was greater than 12,000 mg/m^3. Acute behavioural studies in rats indicated that hypothermia contributed to the behavioural effect of an interperitoneal injection of 800 mg/kg of sulfolane. Rabbits became hyperthermic, at 28°C, upon subcutaneous injection of 600 mg/kg sulfolane.

The chemical is not irritating to guinea pig and rabbit skin or to rabbit eyes. The chemical was not sensitising (0/20) in a guinea pig maximisation test [84/449/EEC, B6].

In a 28 day repeat dose toxicity study [Japanese TG] conducted under GLP, male and female rats were dosed by gavage with this chemical at 0, 60, 200 and 700 mg/kg/day. At 700 mg/kg some females showed transient reduction in locomotor activity during the early administration period. Bodyweight gain and food consumption at this dose were decreased in both males and females. Blood chemistry revealed increases in cholinesterase activity and total bilirubin levels in males and GPT in females and decreases of chloride levels in males and glucose levels in females. Histopathological examination in males dosed at 700 and 200 mg/kg/day revealed increases of hyaline droplets and eosinophilic bodies in the renal tubules which was accompanied by an increase in relative kidney weight. There was a decrease of splenic weight in females at 700 mg/kg/day, but no histological abnormalities were detected. No changes considered to be attributable to sulfolane were observed on urinary and haematological examinations at any dose. Kidney lesions tended to recover and the other changes related to the chemical disappeared after a 14 day recovery period. The NOAEL was 60 mg/kg/day for male rats and 200 mg/kg/day for female rats.

The chemical was not mutagenic in bacteria [OECD TG 471 and 472] and did not induce chromosome aberrations in mammalian cells in vitro [OECD TG 473] either with or without metabolic activation.

In a reproduction/developmental toxicity screening test [OECD 421]) rats were dosed at 0, 60, 200, or 700 mg/kg/day by gavage for 41 to 50 days from 14 days prior to mating to day 3 of lactation. Some mortality occured in the high-dose group. There was a decrease in body weight gain and food consumption of males and females during the pre-mating period, at 700 mg/kg. The number of oestrus cycles was decreased in the 700 mg/kg group. Four dams lost all their pups during the lactation period in the 700 mg/kg group. Birth index, live index, number of pups on days 1 and 4 of lactation, viability index and body weights of pups of both sexes on days 0 and 4 of lactation decreased, and the number of still births increased in the 700 mg/kg group. Birth index

and the number of pups on day 0 and 4 of lactation decreased in the 200 mg/kg group. The NOAEL for reproductive and developmental toxicity was 60 mg/kg/day. There were no treatment-related findings in the external appearance, general conditions and necropsy findings in offspring.

Environment

The chemical has a log Pow of –0.77, a vapour pressure of 0.0083 hPa at 20°C and a water solubility of greater than 100 g/l. Fugacity model Mackay level III calculations suggest that the chemical will distribute almost completely to water if released to the aquatic compartment and equally to soil and water if released into air or soil separately or simultaneously to all three compartments. The chemical is not readily biodegradable (10% after 14 days), and is hydrolytically stable (t1/2 greater than 1 year at pH 4, 7 and 9, 25°C). It can be biodegraded after acclimatisation of activated sludge and by a variety of bacterial cultures, and may be substantially biodegradable. Inorganic sulphate has been identified as the final degradation product of sulfolane metabolism. The chemical has been shown to have low potential for bioaccumulation. Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 9.7 h (calculated using AOPWIN rate constant, 1.328×10^{-11} cm^3/molecule/sec).

In an acute fish toxicity study [OECD TG 203, *Oryzias latipes*] a 96-hLC_{50} > 100 mg/l was reported. In *Daphnia magna* [OECD TG 202], an acute toxicity value of 48h EC_{50} = 852 mg/l was reported. The results in algae [OECD 201] were an E_rC_{50} (72h) >1000 mg/l,E_bC50 = 500mg/ l and a $NOEC_r$ (72 h) = 556 mg/l, $NOEC_b$ = 171 mg/l. The chronic toxicity to *Daphnia magna* [OECD 211] was a NOEC (21d, reproduction) of 25 mg/l and an LC_{50} (21d, parental) > 100 mg/l.

In a study to determine plant toxicity [Environment Canada protocol, lettuce (*Lactuca sativa*), carrot (*Daucus carota*), alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*)] it was determined that plants were generally most sensitive to sulfolane in till and least sensitive in loam. A five day seed germination/root elongation test conducted using lettuce (*Lactuca sativa*) reported NOEC values of 290 mg/kg (root elongation) and 570 mg/kg (seed germination) for lettuce grown in fine-textured soil.

Exposure

Production of the chemical during 2003 was 1100 t/year in Japan. Global production in 2003 was approximately 13,300 t/year. Geographically, production was divided between sites in the Americas (35-45%), Asia (20-30%) and Europe/Africa (35-45%).

The major use of Sulfolane is as a solvent for extraction of aromatic hydrocarbons from oil refinery streams and acid gas purification. These uses account for approximately 80% of production. A number of minor uses (accounting for 20% of production) include fractionation of wood tars, tall oil and other fatty acids, electronic applications, textile manufacturing and finishing, as a plasticizer and as a solvent in pharmaceutical manufacturing. Other uses mentioned in the literature include solvent for jet printing inks, a component of hydraulic fluid, a curing agent for epoxy resins and medicinal application (although this latter application is thought to exist in the patent literature only).

Monitoring studies performed in the vicinity of gas processing facilities in Canada have shown that environmental release of sulfolane during its use in these facilities is possible. Sulfolane was detected in soil, bedrock and shallow till aquifers, wetlands and creeks near these facilities. It was also detected in wetland vegetation.

There is low potential for exposure to workers during production of the chemical. It is manufactured in a closed system and transferred directly from the reactors into storage tanks. There is potential exposure to workers during drum filling. This operation is performed on 16 days per year for 7 hours each day. The concentration of sulfolane close to the drums has been measured at 0.2 ppm. There is potential exposure to workers at user sites. Since the predominant use of sulfolane is as a solvent in commercial extraction processes there is little potential for direct consumer exposure, however there is a potential for indirect human exposure via drinking water and food crops in areas surrounding processing plants.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human health: The chemical is a candidate for further work. The chemical possesses properties indicating a hazard for human health (reproductive and developmental toxicity). Based on data presented by the Sponsor country worker exposure in sites manufacturing the chemical is controlled. No information is available for occupational exposure in industries using the chemical nor for indirect human exposure via drinking water and food crops in areas surrounding processing plants. It is therefore recommended that member countries perform an exposure assessment for industrial users and indirect human exposure, and if then indicated, risk assessments be performed.

Environment: The chemical is currently of low priority for further work because of its low hazard potential.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 126-33-0
IUPAC Name: Tetrahydrothiophene-1,1-dioxide
Molecular Formula: $C_4H_8O_2S$
Structural Formula:

Smiles code C1CCCS1(=O)(=O)
Molecular Weight: 120.17
Synonyms: 1,1-Dioxothiolan; Cyclic tetramethylene sulfone; Cyclotetramethylene sulfone; Sulfolan; Sulfolane; Tetramethylene sulfone.

1.2 Purity/Impurities/Additives

Purity > 99.5% (anhydrous solid grade)

Purity > 95% (liquid grade containing water)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	Colorless solid	
Melting point	27ºC	(Lewis, 2003)
Boiling point	285ºC	(Lewis, 2003)
Vapour pressure	0.0083 hPa at 20 ºC	(Daubert & Danner, 1989)
Water solubility	≥ 100 g/l at 25°C Freely soluble in water	(MITI Japan, 2001)
Partition coefficient n-octanol/water (log value)	-0.77	(Hansch & Leo,1981)
Relative density	1.266 at 30°C	(Lindstrom & Williams, 1983)
Koc (LogKoc)	21.59 (1.334)	(Safepharm, 2004)
Henry's law constant	1.581E-08 atm-m^3/mole	(Safepharm, 2004)

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Production of sulfolane during 2003 was 1100 t/year in Japan. Global production in the rest of the world in 2003 was approximately 13,300 t/year. Geographically, production was divided between sites in the Americas (35-45%), Asia (20-30%) and Europe/Africa (35-45%).

Industrially, sulfolane is produced by hydrogenation of 3-sulfolene ($C_4H_6SO_2$) which is prepared through the reaction of butadiene (C_4H_6) and sulphur dioxide (SO_2).

The major use of Sulfolane is as a solvent for extraction of aromatic hydrocarbons from oil refinery streams and acid gas purification. These uses account for approximately 80% of production. A number of minor uses (accounting for 20% of production) include fractionation of wood tars, tall oil and other fatty acids, electronic applications, textile manufacturing and finishing, as a plasticizer and as a solvent in pharmaceutical manufacturing. Other uses mentioned in the literature include solvent for jet printing inks, a component of hydraulic fluid, a curing agent for epoxy resins and medicinal application (although this latter application is thought to exist in the patent literature only).

In Japan, approximately 400 tons of sulfolane will be transported in drums and approximately 700 tons will be transported in tank cars.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Emission from the Befu Factory of Sumitomo Seika in Japan has been measured as 25 mg/l (Sumitomo Seika, 2004b). This is the only sulfolane production site in Japan. Total wastewater from the plant is approximately 13,000 m^3/year. Wastewater from various plants is combined and mixed with cooling water to make a total volume of wastewater of approximately 14,000,000 m^3/year, which is discharged to the Seto Inland Sea. The main source of pollution is emission in the place of use. However, no information relating to specific sites is available.

2.2.2 Photodegradation

Indirect photo-oxidation by hydroxy radicals (1500000 molecule/cm^3) is predicted to occur with a half-life estimated at 9.7 hours (calculated using AOPWIN rate constant, 1.328 x 10^{-11} cm^3/molecule/sec) (Safepharm, 2004).

2.2.3 Stability in Water

The half-life is >5 days at 50 ºC, pH 4, 7, and 9 (OECD 111) (equivalent to a half-life > one year at room temperature) and sulfolane is considered to be stable to hydrolysis under environmental conditions (MITI Japan, 2001).

2.2.4 Transport between Environmental Compartments

Fugacity Model Mackay level III calculations (Safepharm, 2004) suggest that sulfolane will distribute almost completely to water if released to the aquatic compartment and equally to soil and water if released into air or soil separately or simultaneously to all three compartments.

Table 2 Environmental distribution of sulfolane using Fugacity Model Mackay Level III

	1000 kg/h emission to these compartments separately			1000 kg/h simultaneous emission to air, water & soil
	Air	**Water**	**Soil**	
In air	0.3 %	0.0 %	0.0 %	0.1
In water	50.7 %	99.6 %	45.2 %	57.6
In soil	48.8 %	0.0 %	54.6 %	42.2
In sediment	0.2 %	0.4 %	0.2 %	0.1

2.2.5 Biodegradation

Sulfolane is not readily biodegradable (10% based on BOD after 14 days, Japanese MITI reports) (METI Japan, 1975).

Chou *et al* (1983) investigated the biodegradation of sulfolane in two bench-scale suspended growth systems. A completely mixed activated sludge system (CMAS) was used to simulate a wastewater treatment plant and a continuously stirred tank reactor (CSTR) was used to simulate an aerated lagoon facility. The CMAS reactor was seeded with fresh activated sludge taken from a refinery biotreater and continuously fed with effluent from the same plant (2 l/day). The sludge was acclimated by the addition of 20 mg/l sulfolane to the feed. The activated sludge in the CMAS unit became acclimated to 20 mg/l sulfolane and showed more than 80% sulfolane removal after a week. At this time the CSTR unit was seeded with acclimated sludge from the CMAS. It became effective in sulfolane removal at less than the 2-day hydraulic retention time (approximately 90% removal observed). Inorganic sulfate was identified as the final degradation product of sulfolane metabolism.

Greene et al (2000) investigated the aerobic biodegradation of sulfolane by two mixed microbial enrichment cultures and by three bacterial isolates. Sulfolane served as the sole carbon, sulphur and energy source for these cultures. In the two mixed cultures, 60% and 80% of the sulfolane carbon was recovered as carbon dioxide, whereas in cultures of the three isolates only 40-42% of the substrate carbon was recovered as carbon dioxide. In the mixed cultures, 81% and 97% of the sulfolane sulphur was converted to sulphate, and in the pure isolates, 55-90% of the substrate sulphur was converted to sulphate. Thus, the mixed cultures were capable of greater mineralisation than the pure isolates.

Based on these studies it is considered that Sulfolane may be substantially biodegradable.

2.2.6 Bioaccumulation

Sulfolane had low bioaccumulation characteristics (BCF: High exposure level (2.5 mg/l): <1.3; Low exposure level (0.25 mg/l): <13, Japanese MITI reports) when common carp (*Cyprinus carpio*) were exposed to the chemical for 42 days at 25°C (METI Japan, 1997). BCF of 3.16 was calculated using BCFWIN v2.15 (Safepharm, 2004).

2.2.7 Other Information on Environmental Fate

Data has been collected from 3 sour gas processing facilites in Alberta and British Columbia, Canada (Komex, 2001). At these facilities, a maximum soil sulfolane concentration of 701 mg/kg was measured in clay-rich till. Maximum measured sulfolane concentrations in groundwater collected from aquifers beneath one of the gas processing facilities were 88 mg/l in a bedrock aquifer and 800 mg/l in a shallow till aquifer. At one of the facilities, sulfolane-impacted

groundwater discharged via a wetland into a creek. Levels within the wetland and the creek were significantly reduced compared to the discharging groundwater. Maximum sulfolane concentrations reported in groundwater and creek water were 800 and 0.4 mg/l, respectively.

Sulfolane uptake by wetland vegetation near an industrial gas processing facility in Western Canada was studied (Komex, 2001) as part of a program to evaluate natural attenuation processes in contaminated wetlands. Roots, stems, leaves, flower head, seed heads and berries of cattail, dogwood, sedge, marsh reed grass, cow parsnip and smooth brome growing in sulfolane-impacted wetland were included in the study. Results indicated highly variable sulfolane concentrations for different parts of the same species, between different plant species and between different samples of the same part of the same species. The maximum measured sulfolane concentration in plants from wetland was 256 mg/kg. The maximum measured sulfolane concentration within the wetland was 185 mg/l.

2.3 Human Exposure

2.3.1 Occupational Exposure

There is low potential for exposure to workers during production of sulfolane as the chemical is manufactured in a closed system and transferred directly from the reactors into storage tanks in liquid form. The transfer operation takes approximately 30 minutes for the transfer of 10 tons of sulfolane. No workers are present during this operation. Removal of the filling tube at the end of the transfer takes approximately one minute. There is potential exposure to workers during drum filling. This operation is performed on 16 days per year for 7 hours each day. The concentration of sulfolane close to the drums (0.5m distance) sampling 30 L air has been measured at 0.2 ppm using an impinger (10 mL ethanol) and GC method. The airborne concentration falls to < 0.1 ppm at 3 m from the drums. (Sumitomo Seika, 2004a).

Sampling of sulfolane is performed about 10 times a month. At sampling, which takes approximately three minutes, approximately 500 mL of sulfolane (liquid) are extracted and analysed. Exposure levels during this procedure are not monitored.

No data on exposure from use sites are available.

2.3.2 Consumer Exposure

Since the predominant use of sulfolane is as a solvent in commercial extraction processes there is little potential for direct consumer exposure.

Monitoring studies in Canada (Komex, 2001) suggest that sulfolane may be found in significant amounts in water, ground water and vegetation in areas surrounding processing plants. This indicates a potential for indirect human exposure via drinking water and food crops.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Studies in Animals

In vivo Studies

Groups of 3 male rats were dosed intravenously at 500 or 1000 mg/kg with non-radiolabelled sulfolane and the amount of sulfolane excreted unchanged in the urine was measured for 7 days

after administration using gas-liquid chromatography. At 500 mg/kg, 28%, 36% and 37% of the dose was excreted unchanged between days 0-2, 0-4 and 0-7, respectively. At 1000 mg/kg, 50% and 67.2% of the dose was excreted unchanged between days 0-2 and 0-4, respectively. The observation that the proportion of the dose recovered increased with dosage suggests that the metabolic pathway is saturable. In a follow up to this study, blood-sulfolane decay curves were obtained following intravenous injections of sulfolane to a single rabbit, dog and squirrel monkey. Sulfolane was rapidly distributed throughout the animals and being slowly removed from plasma with a half-life of 3.5-5 hours (Andersen, 1976). No information is given on which tissues sulfolane distributes to in this study.

In a second study 3 male Wistar rats were injected intraperitoneally with ^{35}S-sulfolane (100 mg/rat in 2 ml water) and the 24 hour urinary samples analysed. One major metabolite was found, constituting 85% of the urinary radioactivity. Subsequently, 3 rabbits were injected intraperitoneally with a mixture of unlabelled sulfolane (1g) and ^{35}S-sulfolane (100 mg) and the urine samples collected and extracted with chloroform. The metabolite was identified as 3-hydroxysulfolane (Roberts, 1961).

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

There is one reliable study reported. Andersen et al (1977) examined the acute inhalation toxicity of sulfolane. No rats died after 4 hours exposure to sulfolane at 12,000 mg/m^3 (the highest concentration that could be maintained as a stable aerosol) or during a subsequent 2-week observation period. Exposures at these high concentrations were continued until all all rats died. It was calculated that a mean survival time of 24 hours would be observed in atmospheres containing 4700 mg/m^3 of sulfolane. In a second experiment, nine rats were exposed to sulfolane at a concentration of 3600 mg/m^3 for 17.5 hours (when all the rats had convulsed and were *in extremis*). Significant decreases in white blood cell counts were observed, but haemocrit and haemoglobin were unchanged. At necropsy, all rats exhibited varying degrees of pulmonary haemorrhage. In a third experiment, two squirrel monkeys exposed to 4850 mg/m^3 vomited and convulsed during exposure and were sacrificed after 18.5 hours. Both had a greater than 25% decrease in white blood cells and a greater than 15% reduction in haemoglobin and haematocrit. Once again, pulmonary haemorrhage was evident. The LC_{50} (4h, rat) was > 12,000 mg/m^3.

Table 3 Acute inhalation toxicity to experimental animals

Species	Exposure time	Result	Reference
Rat	4 h	LC_{50} >12 000 mg/m^3 (combined)	(Andersen et al., 1977)
Rat	17.5 h	Total mortality: LC_{50} <3600 mg/m^3 (males)	
Monkey	18.5 h	Total mortality: LC_{50} <4850 mg/m^3 (males)	

Dermal

There are four acute dermal toxicity studies, one of which is considered reliable. In this study, conducted to GLP [Directive 84/449/EEC, B3], sulfolane was applied directly to the intact skin of 5

male and 5 female rats at a dose of 2000 mg/kg and covered with an occlusive dressing for 24 hours. There were no deaths or signs of systemic toxicity during the 14-day observation period and no macroscopic changes were apparent at necropsy (Gardner, 1993). The LD_{50} was > 2000 mg/kg.

Oral

There are 10 acute oral toxicity studies. In a reliable study [OECD TG 401, GLP] male and female rats (5 animals of each sex per dose) were dosed by gavage at doses of 0, 892, 1204, 1626, 2191, 2963 and 4000 mg/kg. Clinical signs of convulsion as well as decreased locomotion activity, ptosis, salivation, piloerection, chromodacryorrhea and perineal region soiling with urine were observed in the treated groups. Body weights of the treated animals were lower than those of the control group on the day after dosing. All deaths occurred on the day of dosing at doses of 2195 mg/kg and above. Dead animals showed haemorrhagic black spots in their glandular stomach mucosa. The LD_{50} was 2006 mg/kg (males) and 2130 mg/kg (females) (MHW Japan, 1996a).

In a second reliable study [Directive 84/449/EEC, B1, GLP] male and female rats (5 animals of each sex per dose) were dosed by gavage at 1600, 2240 and 3136 mg/kg. Deaths occurred from two hours after dosing until Day 2 among rats treated at the intermediate and high dose levels. Clinical signs included fasciculation, tremor, twitching, splayed gait, hunched posture, piloerection, unkempt appearance and yellow staining of the anogenital fur. Convulsions and salivation developed among the rats dosed at 2240 and 3136 mg/kg. Isolated cases of hypersensitivity to stimuli, hyperactivity, lethargy, hypothermia, diarrhoea, lachrymation, pallor of the eyes and blood around the mouth were also observed. Onset of the principal clinical signs was generally apparent within four hours of dosing. All surviving rats had gained weight relative to their Day 1 bodyweights by the end of the of the 14 day observation period. Necropsy findings amongst the decedents were lung congestion, exaggerated lobular pattern or dark patches on the liver, darkening of the spleen or kidneys and abnormal contents (colourless liquid or gaseous) of the gastrointestinal tract, especially the stomach and small intestine. Rats killed at completion of the observation period showed no macroscopic changes other than a single case of hepatic pallor. The LD_{50} was 2489 mg/kg (males), 2324 mg/kg (females) and 2473 mg/kg (combined) (Gardner, 1993).

In a third reliable study male and females rats (5 animals of each sex per dose) were dosed by gavage at 0, 1000, 1500, 2000, 3000 and 5000 mg/kg (males) and 0, 1000, 2000, 2500, 3000 and 5000 mg/kg (females). One male and five females dosed at 1000 mg/kg and four males dosed at 1500 mg/kg appeared normal from normal to termination. Clinical signs noted in the remaining animals included depression, slight depression, rough coat, salivation, hunched appearance, tremors, ataxia, urine stains, soft faeces and red stains on the nose and/or eyes. All surviving rats that showed clinical signs appeared normal by Day 3 through to termination of the study. All surviving rats gained weight relative to their Day 1 bodyweights. No gross pathological findings were observed in rats surviving to termination. Alterations of the stomach and/or intestines were the most common findings amongst animals that died. These alterations included compound like material, dark red material, reddish fluid or yellowish fluid in the stomach and/or intestines. Findings in the lung and liver were noted at the 5000 mg dose only. The LD_{50} was 2739 mg/kg (males), 2108 mg/kg (females) and 2363 mg/kg (combined) (Phillips Petroleum Company, 1983a)

Table 4 Acute oral toxicity in experimental animals

Species	LD_{50}	Reference
Rat	2006 (males); 2130 mg/kg (females)	(MHW Japan, 1996a)
Rat	2489 (males); 2324 (females); 2473 mg/kg (combined)	(Gardner, 1993)
Rat	2739 (males); 2108 (females); 2363 mg/kg (combined)	(Phillips Petroleum Company, 1983a)

Other Routes of Exposure

Ruppert and Dyer (1985) studied the influence of hypothermia on the acute behavioural toxicity of sulfolane. Adult male rats (Long-Evans), 10 per group, received a single interperitoneal injection of saline, 200, 400 or 800 mg/kg sulfolane. Separate groups of rats at each dose were housed in rooms maintained at 32.3±0.7°C (warm ambient temperature) or 20.8±0.2°C (cool ambient temperature). Motor activity was assessed in figure of eight mazes one hour after dosing. Immediately after testing (one hour), body temperatures were recorded.

At the cool ambient temperature, the body temperature of rats receiving 400 and 800 mg/kg was lower than that of the controls. At the warm ambient temperature, hypothermia in the rats receiving 400 and 800 mg/kg was attenuated, if not prevented. One animal receiving 800 mg/kg at the warm ambient temperature died during testing. At both ambient temperatures, 400 and 800 mg/kg sulfolane produced a decrease in motor activity. At the cool ambient temperature, 800 mg/kg sulfolane produced a decrease in movement throughout the maze. It was concluded that a behavioural change could be detected at sublethal dosages of sulfolane in the absence of hypothermia.

Another similar study was conducted by Mohler and Gordon (1988) to investigate the thermoregulatory responses of the rabbit. Nine male rabbits were subcutaneously injected with 0, 100, 200, 400, 600 and 750 mg/kg sulfolane at an ambient temperature of 10°C. This caused a dose-dependent decrease in colonic temperature of the rabbits. Metabolic rate remained unchanged during the initial phase of the hypothermia for all dose groups; but peripheral vasodilation, as indicated by an increase in ear skin temperature, was seen at the higher dose levels. The highest doses of sulfolane caused behavioural deficits in the rabbits. Two to three hours after exposure to 600 mg/kg sulfolane, when the rabbits were removed from the environmental chamber and first observed, the animals exhibited a slight postural tremor similar to shivering. Both rabbits receiving 750 mg/kg sulfolane exhibited tonic seizures characterised by gross muscle contraction, forceful urination, and some vocalisation. These episodes were followed by exhaustion, panting, loss of postural control, and near catatonia. All rabbits in these experiments survived the sulfolane exposure, even at the highest dose levels. Seven male rats were subcutaneously injected with 600 mg/kg sulfolane at ambient temperatures of 10, 20 and 28°C. At ambient temperatures of 10 and 20°C there was a significant decrease in colonic temperature, however metabolic rate did not change significantly prior to or during peak hypothermia. At an ambient temperature of 28°C, there was a significant increase in colonic temperature and metabolic rate following administration of sulfolane.

Conclusion

The oral LD_{50} (rat) was 2006 mg/kg (males) and 2130 mg/kg (females) [OECD TG 401]. The dermal LD_{50} (rat) was > 2000 mg/kg [Directive 84/449/EEC, B3]. The inhalation LC_{50} (4h, rat) was > 12,000 mg/m^3.

Acute behavioural studies indicated hypothermia contributed to the behavioural effect of 800 mg/kg sulfolane in rats, however, the rabbits became hyperthermic, at 28°C, upon injection of 600 mg/kg sulfolane.

3.1.3 Irritation

Skin Irritation

Studies in Animals

In a reliable study (Brown et al., 1966) undiluted sulfolane (1 ml) was applied to the shaved backs of 4 male and 4 female rabbits on 3 consecutive days and covered with an occlusive bandage for 6 hours each day. The final visual assessment was made on day 7. No signs of skin irritation were observed in any of the rabbits used. Histopathological examination of the skins taken post mortem revealed no evidence of skin damage.

Brown et al. (1966) also applied undiluted sulfolane (0.5 ml) daily, five days per week for four and a half weeks to the shaved backs of 10 guinea pigs. Application areas were left uncovered during the test. In findings similar to those in the rabbit, no signs of skin irritation were observed. Histopathological examination of the skins taken post mortem revealed no evidence of skin damage.

Eye Irritation

Studies in Animals

In a reliable study [US Federal Register 29 FR 13009] undiluted sulfolane (0.2 ml) was instilled into the right eyes of rabbits. Only a mild conjunctivitis was produced, which cleared within a few hours (Brown et al., 1966).

Conclusion

Sulfolane is not considered to be a skin or eye irritant.

3.1.4 Sensitisation

Studies in Animals

Skin

There are 3 sensitisation studies, one of which is reliable. In a guinea pig maximisation test [Directive 84/449/EEC, B6, GLP] a group of 10 male and 10 female guinea pigs were induced intradermally using 2% m/v sulfolane in water/Freunds Complete Adjuvant followed a week later by topical induction using undiluted sulfolane (0.3 ml) which was applied over the sites of the intradermal injections and covered occlusively for 48 hours. Challenge was carried out 3 weeks after the intradermal induction. Undiluted sulfolane (0.1ml) was applied to the shaven backs of the test animals and covered with occlusive tape for 24 hours. Dermal reaction to the challenge was assessed after removal of the bandages and at 24 hours and 48 hours after challenge. None of the test animals showed any positive response at either 24 or 48 hours after removal of the challenge patches and therefore sulfolane is not considered to be a skin sensitiser in guinea pigs (Gardner, 1993).

Conclusion

Sulfolane is not considered to be a skin sensitiser in guinea pigs.

3.1.5 Repeated Dose Toxicity

There are 8 studies for repeat dose inhalation toxicity and one study for repeat dose oral toxicity. None of the inhalation studies are considered to be reliable due to the non-standard test methods used. The oral study is considered to be reliable.

Studies in Animals

Oral

In a 28 day repeat dose toxicity study [Japanese TG] conducted to GLP (MHW Japan, 1996b) male and female rats were administered doses of 0, 60, 200 and 700 mg/kg/day of the chemical by gavage. There were 12 animals per dose for the group at 60, 200 mg/kg/day and 24 per dose for the group at 0, 700 mg/kg/day. The recovery period was 14 days.

At 700 mg/kg some females showed transient reduction of locomotor activity at the early stage of the administration period. Bodyweight gain and food consumption at this dose were decreased in both males and females. Blood chemistry revealed increases in cholinesterase and total bilirubin in males and GPT in females and decreases of chloride in males and glucose in females. Pathological examination revealed increases of hyaline droplets and eosinophilic bodies in the renal tubules and an increase in the relative weight of the kidney in males. There was a decrease of splenic weight in females, but no histological abnormalities were detected.

At 200 mg/kg pathological examination revealed increases of hyaline droplets and eosinophilic bodies in the renal tubules of males.

No changes considered to be attributable to sulfolane were observed on urinary and haematological examinations at any dose. Kidney lesions tended to recover and the other changes related to the chemical disappeared after a 14 day recovery period. The NOAEL is considered to be 60 mg/kg/day for males and 200 mg/kg/day for females.

Conclusion

The oral NOAEL is 60 mg/kg/day (males) and 200 mg/kg/day (females).

3.1.6 Mutagenicity

There are 8 *in vitro* mutagenicity studies, seven of which are considered to be reliable. There are no *in vivo* studies available.

In vitro Studies

Sulfolane has been tested for reverse mutation in *Salmonella typhimurium* and *Escherichia coli* with and without exogenous metabolic activation by standard Japanese test methods in full compliance with OECD TG 471 and 472 (MHW, 1996c). No cytotoxicity was observed at 5000 µg/plate in any of the 5 strains. The tests were negative, in both the presence and absence of a metabolising system. An *in vitro* chromosome aberration study in CHL cells was conducted in accordance with Japanese guidelines similar to OECD TG 473 (MHW, 1996d). The highest dose level was cytotoxic. Structural chromosomal aberrations and polyploidy were not induced up to the maximum dose either in the presence or absence of a metabolising system.

Several other bacterial mutagenicity tests, a chromosome aberration study using rat liver RL4, a sister chromatid exchange study and a yeast gene mutation assay are also reported as negative.

In a mouse lymphoma assay (Phillips Petroleum Company, 1982b) exposure to sulfolane in the presence and absence of metabolic activation increased the induction of forward mutations in L5178Y mouse lymphoma cells at the T/K locus. Sulfolane was considered to be mutagenic in this

test system by the authors. However, there was no dose response and the survival percentage was not affected by increasing doses, therefore it is considered that this interpretation of the data is incorrect.

Table 5 Genotoxicity studies of sulfolane

Type of test	Test system	Dose	Result	Reference
Bacterial test (reverse mutation)	*S. typhimurium* TA 98, TA100, TA 1535, TA 1537. *E. coli* WP2uvrA	5 doses between 313 to 5000 µg/plate	Negative, with and without metabolic activation	(MHW, 1996c)
Bacterial test (reverse mutation)	*S. typhimurium* TA 98, TA100, TA 1535, TA 1537, TA 1538. *E. coli* WP2, WP2uvrA	8 doses between 31.25 to 4000 µg/plate	Negative, with and without metabolic activation	(Thorpe, 1982)
Bacterial test (reverse mutation)	*S. typhimurium* TA 98, TA100, TA 1535, TA 1537, TA 1538	5 doses between 642 to 52000 µg/plate	Negative, with and without metabolic activation	(Phillips Petroleum Company, 1982a)
In vitro chromosome aberration assay	CHL/IU	-S9: (continuous exp.24 or 48 h) 0.3, 0.6, 1.2 mg/ml +/- S9: (6h exp.) 0.3, 0.6, 1.2 mg/ml	Negative with and without metabolic activation	(MHW, 1996d)
Sister chromatid exchange	CHO	70, 210, 700, 2100, 6400 µg/ml	Negative with and without metabolic activation	(Phillips Petroleum Company, 1983b)
Yeast gene mutation assay	*Saccharomyces cerevisiae*	0.01, 0.1, 0.5, 1.0, 5.0 mg/ml	Negative with and without metabolic activation	(Thorpe, 1982)
In vitro chromosome aberration assay	Rat liver RL4	250, 500, 1000 µg/ml	Negative without metabolic activation	(Thorpe, 1982)
Mouse lymphoma assay	L5178Y T/K locus	60, 90, 135, 202, 301, 449, 670, 1000 µg/mL	Positive*	(Phillips Petroleum Company, 1982b)

* There was no dose response and the survival percentage was not affected by increasing doses, therefore it is considered that this interpretation of the data is incorrect.

In vivo Studies

No data available

Conclusion

Sulfolane was not mutagenic in bacteria [OECD TG 471 and 472] and did not induce chromosomal aberrations in mammalian cells *in vitro* [OECD TG 473] either with or without metabolic activation.

3.1.7 Carcinogenicity

No data available.

3.1.8 Toxicity for Reproduction

There is one study available for reproductive/developmental toxicity.

Studies in Animals

Effects on Fertility

A reproduction/developmental toxicity screening test [OECD TG 421] was performed (MHW Japan, 1999). Twelve animals of each sex were dosed once daily by gavage (0, 60, 200, 700 mg/kg b.w./day). Males were dosed for 49 days (from 14 days prior to mating) and females for 41-50 days (from 14 days prior to mating to day 3 of lactation). One male and one female in the 700 mg/kg group died. There was a decrease in body weight gain and food consumption amongst males, and females during the pre-mating period, at 700 mg/kg. The number of oestrus cases was decreased in the 700 mg/kg group. Four dams lost all their pups during the lactation period in the 700 mg/kg group. Birth index, live index, number of pups on days 1 and 4 of lactation, viability index and body weights of pups of both sexes on days 0 and 4 of lactation decreased, and the number of still birth increased in the 700 mg/kg group. Birth index and the number of pups on day 0 and 4 of lactation decreased in the 200 mg/kg group. Parental NOAEL was 200 mg/kg/day. NOAEL for offspring was 60 mg/kg/day.

Developmental Toxicity

In the above study, there were no treatment-related findings in the external appearance, general conditions and necropsy findings of the offspring.

Conclusion

The reproductive toxic effects, such as decreased number of oestrus stages and an increased number of litters totally died, in female parents were found at 700 mg/kg bw/day. Developmental toxic effects, such as decreased birth index and number of pups were observed at 200 mg/kg bw/day and higher. The NOAEL for reproductive and developmental toxicity was 60 mg/kg/day. There were no treatment-related findings in the external appearance, general conditions and necropsy findings in offspring.

3.2 Initial Assessment for Human Health

Groups of 3 male rats were dosed intravenously at 500 or 1000 mg/kg of non-radiolabelled sulfolane and the amount of sulfolane excreted unchanged in the urine was measured for 7 days after administration using gas-liquid chromatography. At 500 mg/kg, 28%, 36% and 37% of the dose was excreted unchanged between days 0-2, 0-4 and 0-7, respectively. At 1000 mg/kg, 50% and 67.2% of the dose was excreted unchanged between days 0-2 and 0-4, respectively. The observation that the proportion of the dose recovered increased with dosage suggests that the metabolic pathway is saturable. In a follow up to this study, blood-sulfolane decay curves were obtained following intravenous injections of sulfolane to a single rabbit, dog and squirrel monkey. Sulfolane was rapidly distributed throughout the animals and was slowly removed from plasma with a half-life of 3.5-5 hours.

In a second study 3 male Wistar rats were injected intraperitoneally with ^{35}S-sulfolane (100 mg/rat in 2 ml water) and the 24 hour urinary samples analysed. One major metabolite was found, constituting 85% of the urinary radioactivity. Subsequently, 3 rabbits were injected intraperitoneally

with a mixture of unlabelled sulfolane and 35S-sulfolane (1 g: 100 mg) and the urine samples collected and extracted with chloroform. The metabolite was identified as 3-hydroxysulfolane.

The Oral LD_{50} (rat) was 2006 mg/kg (males) and 2130 mg/kg (females) [OECD TG 401]. The dermal LD_{50} (rat) was > 2000 mg/kg [Directive 84/449/EEC, B3]. The inhalation LC_{50} (4h, rat) was > 12,000 mg/m^3. The chemical is not a skin irritant or eye irritant [US Federal Register 29 FR 13009] or a skin sensitiser in guinea pigs [Directive 84/449/EEC, B6]. The acute behavioural studies showed that hypothermia contributed to the behavioural effect of 800 mg/kg sulfolane in rats, however, the rabbits became hyperthermic, at 28°C, upon injection of 600 mg/kg sulfolane.

Based on the results of a valid repeat dose study [Japanese TG], the NOAEL for repeat dose toxicity (oral) is 60 mg/kg/day (males) and 200 mg/kg/day (females).

Sulfolane was not mutagenic in bacteria [OECD TG 471 and 472] and did not induce chromosomal aberrations in mammalian cells *in vitro* [OECD TG 473]. There is no information on carcinogenicity, however in the absence of significant mutagenic effects *in vitro* there is no immediate concern.

In a reproduction/developmental toxicity screening test [OECD TG 421] males were dosed for 49 days (from 14 days prior to mating) and females for 41-50 days (from 14 days prior to mating to day 3 of lactation) at 0, 60, 200 and 700 mg/kg. One male and one female in the 700 mg/kg group died. There was a decrease in body weight gain and food consumption amongst males, and females during the pre-mating period, at 700 mg/kg. The number of oestrus cases was decreased in the 700 mg/kg group. Four dams lost all their pups during the lactation period in the 700 mg/kg group. Birth index, live index, number of pups on days 1 and 4 of lactation, viability index and body weights of pups of both sexes on days 0 and 4 of lactation decreased, and the number of still births increased in the 700 mg/kg group. Birth index and the number of pups on day 0 and 4 of lactation decreased in the 200 mg/kg group. Parental NOAEL was 200 mg/kg/day. The NOAEL for reproductive and developmental toxicity was 60 mg/kg/day. There were no treatment-related findings in the external appearance, general conditions and necropsy findings of the offspring.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and Chronic Toxicity Test Results

There are a number of studies reported on determination of acute aquatic effects of sulfolane. However many of the study results are reported by a secondary source and have to be considered as unreliable.

Acute Toxicity Test Results

In a reliable acute fish toxicity study [OECD TG 203] *Oryzias latipes* were exposed under semi-static conditions to sulfolane at a nominal concentration of 0 and 100 mg/L for 96 hours. There were no mortalities or signs of toxicity during the study in either the control or test fish. The LC_{50} was > 100 mg/L. In a further reliable acute fish toxicity study (Stephenson, 1982), *Salmo gairdneri* were exposed under semi-static conditions to sulfolane at concentrations of 100-1000 mg/L for 96 hours. 4 out of 10 fish exposed to 1000 mg/L had died after 96-hours exposure. The LC_{50} was approximately 1000 mg/L.

In an acute aquatic invertebrate toxicity study [OECD TG 202], *Daphnia magna* were exposed under static conditions to sulfolane at concentrations of 0, 95.3, 171, 309, 556 and 1000 mg/L for 48 hours. The percentage swimming inhibition in each test concentration medium was calculated

from the number of daphnia inhibited from swimming and the number of Daphnia used in the test. The median swimming inhibition concentration was calculated by the Probit method. The EC_{50} (immobilisation) was 852 mg/L.

In a reliable study [OECD TG 201], *Selenastrum capricornutum* were exposed under static conditions to sulfolane at nominal concentrations of 0, 95.3, 171, 309, 556 and 1000 mg/L. The E_rC_{50} (24-72h) was > 1000 mg/L.

Table 6 Acute toxicity studies in aquatic organisms

Organism	Duration (h)	Result (mg/L)	Reference
Fish			
Oryzias latipes	96 (ss)	LC_{50} > 100 (nc*)	(Environment Agency Japan, 1999b)
Salmo gairdneri	96 (ss)	LC_{50} ca. 1000; LC_0 350 (nc)	(Stephenson, 1982)
Invertebrates		Immobility	
Water flea (*Daphnia magna)*	48 (s)	EC_{50} 852 (nc*); NOEC 171 (nc*)	(Environment Agency Japan, 1999a)
Water flea (*Daphnia magna)*	48 (s)	EC_{50} (24-hr) 160; EC_{50} (48-hr) 95; EC_0 20, EC_{100} 1000 mg/l (nc)	(Stephenson, 1982)
Aquatic Plants			
Green algae (*Selenastrum capricornutum)*	72 (s)	Biomass: E_bC_{50} (0–72 h) 500 (nc*) Growth rate: E_rC_{50} (24-72 h) > 1000 (nc*)	(Environment Agency Japan, 1999c)
Green algae (*Selenastrum capricornutum)*	96 (s)	EC_{50} > 1000 (nc)	(Stephenson, 1982)

(s): Static, (ss): semi-static

nc: calculated based on nominal concentration

nc*: calculated based on nominal concentration because measured concentration were >80% of nominal concentrations.

Chronic Toxicity Test Results

In a chronic toxicity study [OECD TG 211], *Daphnia magna* were exposed under semi-static conditions to sulfolane at concentrations of 0, 25, 50 and 100 mg/l (Environment Agency Japan, 1999d). EC_{50} (21 days, reproduction) > 100 mg/L, NOEC (21d, reproduction) = 25 mg/L and LC_{50} (21 days, parental) >100 mg/L.

In a reliable study [OECD TG 201], *Selenastrum capricornutum* were exposed under static conditions to sulfolane at nominal concentrations of 0, 95.3, 171, 309, 556 and 1000 mg/L. The NOECr (24-72 h) = 556 mg/L.

Table 7 Chronic toxicity studies in aquatic organisms

Organism	Duration (h)	Result (mg/L)	Reference
Aquatic Plants			
Green algae (*Selenastrum capricornutum)*	72 (s)	Biomass: $NOEC_b$ (0-72 h) 171 (nc*) Growth rate: $NOEC_r$ (24-72h) 556 (nc*)	(Environment Agency Japan, 1999c)
Green algae (*Selenastrum capricornutum)*	96 (s)	NOEC 1000	(Stephenson, 1982)
Invertebrates			
Water flea (*Daphnia magna)*	21 days (ss)	Parental mortality: LC_{50} (21d) > 100 (nc*) Reproduction: EC_{50} (21d) > 100 (nc*) NOEC (21d) 25 (nc*)	(Environment Agency Japan, 1999d)

(s): Static, (ss): semi-static nc*: calculated based on nominal concentration because measured concentration were >80% of nominal concentrations.

Toxicity to Microorganisms

No data available.

4.2 Terrestrial Effects

Two studies are available for the toxicity of sulfolane to terrestrial plants. The first study was conducted on lettuce (*Lactuca sativa*) and consisted of a five day seed germination/root elongation test. NOEC values of 290 mg/kg (root elongation) and 570 mg/kg (seed germination) were reported for lettuce grown in fine-textured soil (Komex 2001). In the second study, conducted using an Environment Canada draft protocol, four plant species (lettuce (*Lactuca sativa*), carrot (*Daucus carota*), alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*)) and four soils with differing texture, organic carbon content and cation exchange capacity were examined. The endpoints measured were emergence, biomass, root length, and shoot length. For lettuce the most sensitive endpoints were emergence and root length (NOEC = 440 mg/kg). For carrot and alfalfa the most sensitive endpoint was root length (NOEC = 440 and 235 mg/kg, respectively). For Timothy, biomass was the most sensitive endpoint, NOEC = 228 mg/kg. Plants were generally most sensitive to sulfolane in till and least sensitive in loam (Komex 2001).

Table 8 Minimum toxicity values for plants exposed to sulfolane

Plant Species	Minimum toxicity values (mg/kg)				Reference
	NOEC	LOEC	EC25	EC50	
Lettuce (*Lactuca sativa*)	290 (root elongation, till)	570 (root elongation, till)	1300 (root elongation, till)	1800 (germination, till)	Komex (2001)
Lettuce (*Lactuca sativa*)	440 (emergence, root length, till)	911 (root length, shoot length, sand)	462 (biomass, artificial soil)	1070 (root length, sand)	Komex (2001)
Carrot (*Daucus carota*)	440 (root length, till)	911 (root length sand)	512 (root length sand)	1800 (root length sand)	Komex (2001)
Alfalfa (*Medicago sativa*)	235 (root length, till)	440 (root length, till)	490 (root length, till)	1530 (root length, till)	Komex (2001)
Timothy (*Phleum pratense*)	228 (biomass, sand)	455 (biomass, sand)	384 (biomass, sand)	911 (root length, sand)	Komex (2001)

4.3 Other Environmental Effects

No data available

4.4 Initial Assessment for the Environment

Sulfolane has a log P_{ow} of –0.77 at room temperature, a vapour pressure of 0.0133 hPa at 20°C and a water solubility of ≥ 100 g/l at 25°C. Fugacity model Mackay level III calculations suggest that the chemical will distribute almost completely to water if released to the aquatic compartment and equally to soil and water if released into air or soil separately or simultaneously to all three compartments.

Sulfolane is not readily biodegradable (10% after 14 days), and is hydrolytically stable (t1/2 greater than 1 year at pH 4, 7 and 9, 25°C). It can be biodegraded after acclimatisation of activated sludge and by a variety of bacterial cultures, and may be substantially biodegradable. Sulfolane can be predicted not to bioaccumulate based on results of bioaccumulation tests (BCF: High exposure level (2.5 mg/L) < 1.3, Low exposure level (0.25 mg/L) < 13).

Reliable acute toxicity data are available for one species of fish (*Oryzias latipes*, LC_{50} > 100 mg/L. In *Daphnia magna*, an acute toxicity value of EC_{50} (48 h) = 852 mg/L and NOEC (48 h) = 171 mg/L) were reported. The chronic toxicity data for *Daphnia magna* were EC_{50} (21 days, reproduction) > 100 mg/L, NOEC (21d, reproduction) = 25 mg/L and LC_{50} (21 days, parental) >100 mg/L. The results in algae were E_rC_{50} (24-72h) > 1000 mg/L, $NOEC_r$ (24-72 h) = 556 mg/L.

Signs of toxicity were noted at high sulfolane concentrations in fish > 1000 mg/L and Daphnia >100 mg/L.

Four plant species were tested for toxicity with sulfolane (lettuce (*Lactuca sativa*), carrot (*Daucus carota*), alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*)). The most sensitive endpoints for lettuce were emergence and root length (NOEC = 440 mg/kg). For carrot and alfalfa the most sensitive endpoint was root length (NOEC = 440 and 235 mg/kg, respectively). For Timothy, biomass was the most sensitive endpoint, NOEC = 228 mg/kg. Plants were generally most sensitive to sulfolane in till and least sensitive in loam. A five day seed germination/root elongation

test conducted using lettuce (*Lactuca sativa*) reported NOEC values of 290 mg/kg (root elongation) and 570 mg/kg (seed germination) for lettuce grown in fine-textured soil.

No data for microorganisms are available

5 RECOMMENDATIONS

Environment:

The chemical is currently of low priority for further work because of its low hazard potential.

Human health:

The chemical is a candidate for further work. Sulfolane possesses properties indicating a hazard for human health (reproductive and developmental toxicity). Based on data presented by the Sponsor country, worker exposure in sites manufacturing the chemical is controlled. No information is available for occupational exposure in industries using the chemical or for indirect human exposure via drinking water and food crops in areas surrounding processing plants. It is therefore recommended that member countries perform an exposure assessment for industrial users and indirect human exposure, and if then indicated, risk assessments be performed.

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I U C L I D

Data Set

Existing Chemical	:	ID: 126-33-0
CAS No.	:	126-33-0
EINECS Name	:	tetrahydrothiophene 1,1-dioxide
EC No.	:	204-783-1
Molecular Formula	:	C4H8O2S

Producer related part

Company	:	Safepharm Laboratories
Creation date	:	07.01.2004

Substance related part

Company	:	Safepharm Laboratories
Creation date	:	07.01.2004
Status	:	
Memo	:	ICCA HPV Sulfolane Sumitomo Seika
Printing date	:	23.07.2004
Revision date	:	
Date of last update	:	15.01.05
Number of pages	:	94
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Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

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Type	:	manufacturer
Name	:	CHEVRON PHILLIPS Chemical Company LP
Contact person	:	Vicente Santa Cruz, Ph.D. Product Stewardship, Toxicology
Date	:	22.07.04
Street	:	10001 Six Pines Drive, Suite 4103
Town	:	The Woodlands, TX 77380
Country	:	USA
Phone	:	+1 832-813-4787
Telefax	:	+1 832-813-4435
Telex	:	
Cedex	:	
Email	:	SANTAV@cpchem.com
Homepage	:	

22.07.2004

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	Sulfolane
Smiles Code	:	C1CCCS1(=O)(=O)
Molecular formula	:	C4H8O2S
Molecular weight	:	120.17
Petrol class	:	

26.03.2004

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	solid
Purity	:	> 99.5 % w/w
Colour	:	Colourless
Odour	:	
Remark	:	Stated purity is for the anhydrous solid grade of material. A liquid grade containing < 5% w/w water is also available.

22.04.2004

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1,1-Dioxothiolan

26.03.2004

Cyclic tetramethylene sulfone

26.03.2004

Cyclotetramethylene sulfone

26.03.2004

Sulfolan

26.03.2004

Sulfolane

26.03.2004

Tetrahydothiophene 1,1-dioxide

26.03.2004

Tetramethylene sulfone

26.03.2004

1.3 IMPURITIES

Purity type	:	typical for marketed substance
Chemical name	:	Related Sulfones
Value	:	< 0.5 % w/w

24.09.2004

Purity type : typical for marketed substance
Chemical name : Sulfides
Value : Trace levels
24.09.2004

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : 1100 - tonnes produced in 2003

Remark : The quantity is the estimated production in Japan
Reliability : (1) valid without restriction
Information provided by sales/marketing department of co-operating company
04.05.2004 (41)

Quantity : 13,300 - tonnes produced in 2003

Remark : The quantity is the estimated global production of sulfolane.
Breakdown by geographic area is estimated as:

35-45% Americas
35-45% Europe/Africa
20-30 % Asia

Reliability : (1) valid without restriction
Information provided by sales & marketing departments of co-operating companies.
04.05.2004 (41)(6)

1.6.1 LABELLING

From the MSDS of Sigma-Aldrich
Section 15 - Regulatory Information
EU DIRECTIVES CLASSIFICATION
Symbol of Danger: Xn
Indication of Danger: Harmful.
R: 22
Risk Statements: Harmful if swallowed.
S: 25 23
Safety Statements: Avoid contact with eyes. Do not breathe fumes.

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Harmful.
Risk Statements: Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.
US Statements: Target organ(s): Blood.
UNITED STATES REGULATORY INFORMATION
SARA LISTED: No
TSCA INVENTORY ITEM: Yes

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: Yes
NDSL: No

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

MAJOR USES (APPROXIMATELY 80% OF TOTAL PRODUCTION)

1. SOLVENT FOR EXTRACTION OF AROMATIC HYDROCARBONS FROM OIL REFINERY STREAMS
2. ACID GAS PURIFICATION

MINOR USES (APPROXIMATELY 20% OF TOTAL PRODUCTION)

1. FRACTIONATION OF WOOD TARS, TALL OIL AND OTHER FATTY ACIDS
2. ELECTRONIC APPLICATIONS
3. TEXTILE MANUFACTURE/FINISHING
4. PLASTICISER
5. SOLVENT USED FOR SYNTHESIS IN PHARMACEUTICAL INDUSTRY

OTHER USES (THESE ARE MENTIONED IN THE LITERATURE, BUT DO NOT NECESSARILY APPLY TO THE PRODUCERS

1. SOLVENT FOR JET PRINTING INKS
2. COMPONENT OF HYDRAULIC FLUID
3. CURING AGENT FOR EPOXY RESINS
4. MEDICINAL APPLICATIONS

1.7.1 DETAILED USE PATTERN

Industry category	:	2 Chemical industry: basic chemicals
Use category	:	48 Solvents
Extra details on use category	:	No extra details necessary No extra details necessary
Emission scenario document	:	not available
Product type/subgroup	:	
Tonnage for Application	:	
Year	:	
Fraction of tonnage for application	:	
Fraction of chemical in formulation	:	
Production	: :	
Formulation	: :	
Processing	: :	
Private use	:	
Recovery	:	

26.03.2004

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information :

26.03.2004

Type : TSCA
Additional information : EPA TSCA Test submission (TSCATS) database, December 1999

26.03.2004

Type : DSL
Additional information : Canadian Inventory

26.03.2004

Type : AICS
Additional information : Australian Inventory

26.03.2004

Type : ECL
Additional information : Korean Inventory of Chemicals

26.03.2004

Type : ENCS
Additional information : Japanese Inventory

26.03.2004

Type : CHINA

Additional information : Inventory of Existing Chemical Substances in China

26.03.2004

Type : PICCS
Additional information : Philippine Inventory

26.03.2004

Type : other: DENMARK
Additional information : The Danish Product Register

26.03.2004

Type : other: SWEDEN
Additional information : The Swedish Product Register

26.03.2004

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure: Human: exposure of the operator
Exposure to the: Substance

Method: Sampling was performed at a factory in Japan manufacturing sulfolane. Approximately 30 L of air from each sampling point was drawn through a liquid trap. Samples were analysed using GC.

Result: The chemical is manufactured in a closed system and transferred directly from the reactors into storage tanks in liquid form. The transfer operation takes approximately 30 minutes for the transfer of 10 tons of sulfolane. No workers are present during this operation. Removal of the filling tube at the end of the transfer takes approximately one minute. There is potential exposure to workers during drum filling. This operation is performed on 16 days per year for 7 hours each day. The concentration of sulfolane close to the drums has been measured at 0.2 ppm using a GC method. At distances of 3 and 5 metres from the drum filling operation, the level of sulfolane was < 0.1 ppm. Sampling of sulfolane is performed about 10 times per month. At sampling, which takes approximately three minutes, approximately 500 mL of sulfolane (liquid) are extracted and analysed. Exposure levels during this procedure are not monitored.

Reliability: (1) Valid without restriction
Well reported study conducted by the manufacturer

Flag: Critical study for SIDS endpoint

24.0.2004 (41)

Source of exposure: Human: exposure of the operator
Exposure to the: Substance

Method: Chevron Phillips has recently performed limited occupational exposure monitoring during sulfolane production and distribution in their Borger, Texas

Facility in the US. Full shift exposure monitoring for four individuals performing capping and sealing of approximately 150 drum orifices (identified as the most likely source of exposure) was performed.

Result: The concentration of sulfolane in the air was below the limit of detection (<0.083 ppm).

Reliability: (4) Not assignable
Limited information available on the method followed.

24.0.2004 (7)

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered :
Date of search : 12.01.2004
Remark : Aquire (1992 - 2002)
Biodegradation Data (BIODEG) (1992 - 2002)
Biodegradation Bibliographic References (BIOLOG)(1992 - 2002)
Biological Abstracts - BIOSIS (1969 - present)
CA Search (1967 - present)
ECOTOX database
EMBASE (1974 - present)
EMBSINFO (1977 - present)
Enviroline (1970 - present)
Environmental Bibliography (1974 - present)
Gene-Tox (1992 - 2002)
HSELINE (1977 - present)
IRIS database
Medline (1966 - present)
National Technical Information Service (NTIS)(1964 - present)
NIOSH (1973 - present)
PASCAL (1984 - present)
TERRETOX (1992 - 2002)
TSCATS (1977 - present)
Toxfile (1965 - present)
Internet

Search terms:

CAS No. 126-33-0
Sulfolane
Ecotoxicology
Toxicology
Environment

04.05.2004

1.13 REVIEWS

2.1 MELTING POINT

Value	:	27 °C
Sublimation	:	
Method	:	other: not specified
Year	:	2003
GLP	:	no data
Test substance	:	
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Peer-reviewed literature data
Flag	:	Critical study for SIDS endpoint
22.06.2004		(19)

Value	:	28.5 °C
Sublimation	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Peer-reviewed literature data
22.06.2004		(20)

2.2 BOILING POINT

Value	:	285 °C at
Decomposition	:	
Method	:	
Year	:	2003
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Peer-reviewed literature data
Flag	:	Critical study for SIDS endpoint
22.06.2004		(19)

Value	:	287.3 °C at
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Peer-reviewed literature data
22.06.2004		(20)

2.3 DENSITY

Type : density
Value : 1.2606 g/cm³ at 30 °C
Method :
Year :
GLP : no data
Test substance :

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Peer-reviewed literature data
22.06.2004 (46)

Type : relative density
Value : 1.266 at 30 °C
Method : other: not specified
Year :
GLP : no data
Test substance :

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Peer-reviewed literature data
22.06.2004 (20)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .0133 hPa at 20 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance :

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Peer-reviewed literature data
22.06.2004 (38)

Value : 0.0083 hPa at 20°C
Decomposition :
Method :
Year : 1991
GLP : no data
Test substance :
Result : Result quoted in the EPIWIN experimental database is 0.0062 mmHg

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) Valid with restrictions
Peer-reviewed literature data
Flag : Critical study for SIDS endpoint
26.09.2004 (10)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	-.77 at °C
pH value	:	
Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Peer-reviewed literature data
Flag	:	Critical study for SIDS endpoint

22.06.2004 (15)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	>= 100 g/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	miscible
Stable	:	
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	1999
GLP	:	no data
Test substance	:	other TS
Method	:	Test material (0.1g) and purified water (1 mL) were placed into a test vessel and shaken. After shaking, the test solution was macroscopically observed for undissolved test material.
Result	:	The test material was completely dissolved and, therefore, the solubility of the test material in water was identified as >= 100 g/L.
Test substance	:	Name: Sulfolane (CAS No. 126-33-0) Purity: 99.6% Lot No.: M6E9460 Supplier: Nacalai Tesque Inc.
Reliability	:	(2) valid with restrictions No information on whether test was conducted to GLP available
Flag	:	Critical study for SIDS endpoint

22.06.2004 (28)

2.6.2 SURFACE TENSION

Test type	:	
Value	:	35.5 mN/m at 30 °C
Concentration	:	
Method	:	other: not specified
Year	:	
GLP	:	no data

Test substance : as prescribed by 1.1 - 1.4

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Not clear whether measurement was made on an aqueous solution or the liquid form of the substance
22.06.2004 (20)

2.7 FLASH POINT

Value : °C
Type : other: not specified
Method : other: not specified
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Flash point = 165 - 178°C
Reliability : (2) valid with restrictions
Peer-reviewed literature data
22.06.2004 (20)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	.0000000000132785 cm³/(molecule*sec)
Degradation	:	50 % after 9.7 hour(s)
Deg. product	:	
Method	:	other (calculated): AOPWIN v1.90
Year	:	2004
GLP	:	
Test substance	:	Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions The value is estimated with the method recommended in the OECD Guidance
Flag	:	Critical study for SIDS endpoint

16.06.2004 (35)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1000000
Rate constant	:	.0000000000045 cm³/(molecule*sec)
Degradation	:	50 % after 1.8 day(s)
Deg. product	:	
Method	:	OECD Guide-line draft "Photochemical Oxidative Degradation in the Atmosphere"
Year	:	1991
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Calculated with the Atkinsons method
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	> 5 day(s) at 50 °C
t1/2 pH7	:	> 5 day(s) at 50 °C
t1/2 pH9	:	> 5 day(s) at 50 °C
Deg. product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	2001
GLP	:	yes
Test substance	:	

Method : Conditions for preliminary test:
Concentration of test material: Approx. 1000 mg/l
Test temperature: 50 +/- 1 degree C
pH of test solution: 4, 7, 9
Test vessel: Stoppered glass flask
No. of replicates: 2
Test period: 5 days

Remark : No hydrolysis at pH 4, 7 and 9 at 50°C for 5 days. Based on the results the substance is not considered to hydrolyse at room temperature at pH 4, 7 or 9. The t1/2 is greater than one year at room temperature at pH 4, 7 and 9. Sulfolane is considered to be stable to hydrolysis under environmental conditions.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 99.6%
Lot No.: M6E9460
Supplier: Nacalai Tesque Inc.

Reliability : (1) valid without restriction
The data are approved by the Japanese government

Flag : Critical study for SIDS endpoint

23.06.2004 (28)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : concentration in wastewater
Media :
Concentration : 25 mg/L
Method :

Remark : Data collected at manufacturing site in Japan. Total wastewater from the plant is approximately 13,000 t/year.

Reliability : (1) valid without restriction
Information provided by manufacturer

24.05.2004 (41)

Type of measurement : concentration at contaminated site
Media :
Concentration :
Method :

Remark : Data collected from 3 sour gass processing facilites in Alberta and British Columbia, Canada. At these facilities, a maximum soil sulfolane concentration of 701 mg/kg was measured in clay-rich till. Maximum measured sulfolane concentrations in groundwater collected from aquifers beneath one of the gas processing facilities were 88 mg/L in a bedrock aquifer and 800 mg/L in a shallow till aquifer. At one of the facilities, sulfolane impacted groundwater discharged via a wetland into a creek. Levels within the wetland and the creek were significantly reduced compared to the discharging groundwater. Maximum sulfolane concentrations reported in groundwater and creek water were 800 amd 0.4 mg/L, respectively.

Sulfolane uptake by wetland vegetation was studied as part of a program to

evaluate natural attenuation processes in contaminated wetlands. Roots, stems, leaves, flower head, seed heads and berries of cattail, dogwood, sedge, marsh reed grass, cow parsnip and smooth brome growing in sulfolane-impacted wetland were included in the study. Results indicated highly variable sulfolane concentrations for different parts of the same species, between different plant species and between different samples of the same part of the same species. The maximum measured sulfolane concentration in plants from wetlans was 256 mg/kg. The maximum measured sulfolane concentration within the wetland was 185 mg/L.

Reliability : (1) valid without restriction
Study reported in peer reviewed literature

04.05.2004 (18)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media :
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Fugacity Model Level III
Year : 2004

Test condition : Inputs:

Molecular weight: 120.17
Melting point: 27
Vapour pressure: 0.0082 Pa
Water solubility: 100000 g/m3
Log Kow: -0.77
Temperature: 25°C
T1/2 (air): 9.7 hours (estimated)
T1/2 (water): 240000 hours (estimated)
T1/2 (soil): 240000 hours (estimated)
T1/2 (sediment): 240000 hours (estimated)

Note: The half life in air was estimated using AOPWIN v 1.90. The default values applied for the other half lives were recommended by CERI, Japan

	1000 kg/h emission to these compartments separately			1000 kg/h simultaneous emission to air, water & soil
	Air	**Water**	**Soil**	
In air	0.3 %	0.0 %	0.0 %	0.1
In water	50.7 %	99.6 %	45.2 %	57.6
In soil	48.8 %	0.0 %	54.6 %	42.2
In sediment	0.2 %	0.4 %	0.2 %	0.1

Conclusion : The chemical will distribute almost completely to water if released to the aquatic compartment and equally to soil and water if released into air or soil separately or simultaneously to all three compartments.

Reliability : (2) valid with restrictions
Estimated using Epiwin software

Flag : Critical study for SIDS endpoint
22.06.2004 (35)

Type : adsorption
Media : water - soil
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: calculation
Year : 2004

Method : Calculated using PCKOCWIN v1.66 (EPIWIN v3.12)
Result : PCKOC Program (v1.66) Results:
==========

Koc (estimated): 21.6

SMILES : O=S(=O)(CCC1)C1
CHEM : Thiophene, tetrahydro-, 1,1-dioxide
MOL FOR: C4 H8 O2 S1
MOL WT : 120.17
---------- PCKOCWIN v1.66 Results----------

First Order Molecular Connectivity Index ..... : 3.207
Non-Corrected Log Koc ..... : 2.3288
Fragment Correction(s):
1 Sulfone (-C-SO2-C-) ..... : -0.9945
Corrected Log Koc ..... : 1.3343

Estimated Koc: 21.59

Reliability : (2) valid with restrictions
Estimated using Epiwin software
07.01.2005 (50)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 1991

Result : Result: According to the Mackay Level I calculation the environmental distribution will be:

air: 0%
water: 100%
Soil/sediment: 0%
Suspended matter: 0%
Biota: 0%

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
22.06.2004 (16)

Media :

Method : other (calculation)
Year : 2004

Result : Result: According to the HENRYWIN v3.10 calculation, Henry's Law constant will be: 1.581E-08 atm-m3/mole
Reliability : (2) valid with restrictions
Calculated using EPIWIN v3.12
Calculated using a water solubility of 100 g/L, which was maximum concentration tested in the water solubility test.
22.06.2004 (35)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time : 14 day(s)
Degradation : 10 (±) % after 14 day(s)
Result : under test conditions no biodegradation observed
Control substance : Aniline
Kinetic : %
%
Deg. product : Not measured
Method : MITI (I) test (OECD TG 301C)
Year : 1975
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : MITI biodegradation. Studies conducted in accordance with generally accepted principles.

Analytical Conditions
Total organic carbon analyser (TOC meter)
Flow rate in TC circuit: 210 ml/min
Temperature in TC furnace: 890 degree C

Gas chromatograph (GC) Detector FID
Carrier gas: N2
Filler: 20% PEG 20M/Chromosorb W
Glass column: 2 mmø x 1 m
column temperature: 182 degrees C

Remark : The percentage biodegradation of the test material was apparently 10% in the indirect determination, but this is attributable to the low volume of basal respiration. The test material showed no biodegradability in the direct determination.
Result : 10% biodegradation after 14 days (based on BOD)
0% biodegradation after 14 days (based on TOC)

The test material is not readily biodegradable.
Reliability : (1) valid without restriction
The data are approved by the Japanese government
Flag : Critical study for SIDS endpoint
23.06.2004 (21)

Type	:	aerobic
Inoculum	:	other: activated sludge taken from a refinery biotreater
Deg. product	:	
Method	:	other: Refinery Wastewater Simulation test
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Analysis:

Analytical conditions
Varian 3700 GC and Vista 401 Integrator
Column: 4 ft, 5% Carbowax 2M on 60-80 mesh Chromosorb T
Temperature: 165 degree C for 9 minutes and then increased to 185 degree C at 10 degree C/minute.
Injector and detector temperature maintained at 225 degrees C.

All oxygen uptake tests were performed with a 250 mL BOD bottle and an Orbisphere oxygen probe equipped with an adapter. Endogenous rates were taken after 30 minutes aeration without feed. When substrate was included, the sludge was washed twice with 10 or 20 mM pH 7 phosphate buffer.

BOD and COD (dichromate) analyses were performed according to Standard Methods (American Public Health Association (1975) Standard Methods for the Examination of Water and Wastewater, 14th ed. Washington DC). TOC was analysed with a Beckman Tocamaster Model 915-B. Sulfate was determined by ion exchange chromatography (Dionex).

Bench-Scale Completely Mixed Activate Sludge system (CMAS):
The bench CMAS system was employed to simulate wastewater treatment operations. The laboratory unit comprised a 3L cylindrical aerator, a 1L clarifier and a variable speed sludge recycle pump. Aeration and mixing were provided with a bottom air diffuser and an impeller. Dissolved oxygen was maintained at 3-6 mg/L and the temperature at 75±5°F. Feed and effluent were refrigerated and the feed flow rate adjusted to 2-4 L/day. Daily effluent composite samples were used for routine BOD, COD, sulfolane and sulfate analysis.

Bench-Scale Continuously Stirred Tank Reactor (CSTR):
This unit was used for the simulation of sulfolane degradation in an aerated lagoon facility with approximately a 2-day hydraulic detention time operated at 75±5°F. The reactor is similar to the CMAS system, minus the clarifier and sludge recycle line. Feed and effluent storage, pH control were conducted as for the CMAS unit.

Batch Die-Away Biodegradation Tests:
Degradation of sulfolane by activated sludge was performed in a 1L cylinder with bottom diffusers. The activated sludge was taken from the CMAS reactor, settled decanted, washed with 20 mM pH 7.2 phosphate buffer and resuspended in the same buffer solution. Sulfolane was added at 100 mg/L and the removal of the compound was analyzed over several days.

Result : Sulfolane can be biodegraded by activated sludge cultures from refinery biotreaters. A short term acclimatization period is required in an activated sludge system or an aerated lagoon system for effective removal of 80 mg/l sulfolane.
Sulfate formation:
During some batch degradation and oxygen uptake tests, it was observed

that there was a significant decrease of pH accompanying sulfolane disappearance. Since inorganic sulfate seemed to be the most probable end product, sulfate and sulfolane analyses and balances were made. The data shown in tables 1 and 2 clearly indicate that the sulfur in sulfolane is stoichiometrically converted to inorganic sulfate through biological oxidation. In most of the batch and continuous flow studies, 95-102% of the sulfur was recovered. No sulfite was observed in effluents from the continuous-flow reactors or in batch die-away tests. It is possible, however, that sulfite may have been oxidised spontaneously and/or intracellularly to sulfate.

CMAS Reactor:
Over a 2-week period prior to sulfalane addition the reactor performed very well and consistently produced an effluent with average 2 mg/L soluble BOD. Following the addition of 20 mg/L sulfolane into the feed, a few days acclimation was required before sulfolane was almost totally removed. The highly efficient BOD removal (effluent soluble BOD = 1 mg/L) indicated that the CMAS aquired its sulfolane degrading capability at no expense to its normal organic removing capacity.

Similar performance results were also obtained when sulfolane was step-increased to 40 and 80 mg/L. Most of the daily effluent composites had no detectable sulfolane (less than 1 mg/L).

CSTR Unit:
The bench-scale CSTR was seeded with preacclimated biomass from the CMAS reactor. At 20 mg/L feed rate, removal of sulfolane and soluble BOD were about 90 and 97%, respectively at hydraulic loadings of 1.42-2.1 days. When influent sulfolane was increased to 40 and 80 mg/L (hydraulic detention time 2.4 and 2.0 days, repsectively) there was no reduction in sulfolane removal efficiency. The effluent sulfolane never exceeded 6 mg/L and averaged 1-2 mg/L for these two test conditions. The average soluble BOD was less than 5 mg/L.

Conclusions : Inorganic sulfate has been identified as a degradation product of sulfolane metabolism.

The addition of sulfolane (up to 80 mg/l) to refinery wastewater does not affect the general performance of the two biological treatment processes studied.

The test material is inherently biodegradable

Table 1: Degradation of Sulfolane: Batch Die-away Test

Reaction Time (hr)	pH	Sulfolane (mg/L)	TOC (mg/L)	SO_4^{2-} (mg/L)	Sulfur Recovery as SO_4^{2-} (%)
0	7.2	100	48	58	-
24	7.0	<1	9	137	98.8[a]
48	7.0	<1	6	140	102[a]

[a] Total degradation of sulfolane is assumed

Table 2: Distribution of Organic and Inorganic Sulfur in the Activated Sludge Reactor

Test No.	Influent Sulfur (mg/L)			Effluent Sulfur (mg/L)			Sulfur Recovery (%)
	Organic-S	SO_4^{2-}-S	Total[a]	Organic-S	SO_4^{2-}-S	Total[a]	
1	12.1	88	100.1	ND[b]	114	114	88
2	12.1	95	107.1	ND	113	113	95
3	24.2	80	104.2	ND	107	107	97
4	24.2	81	105.2	ND	109	109	97

[a] Assuming any other influent sulfur is negligible
[b] ND: Nondetectable or less than 1 mg/L.

Reliability : (2) valid with restrictions
Study reported in peer reviewed literature
Flag : Critical study for SIDS endpoint
23.06.2004 (8)

Type : aerobic
Inoculum :
Deg. product :
Method : other: original MITI
Year :
GLP :
Test substance : as prescribed by 1.1 - 1.4

Remark : sulfolane is listed as a not easily degradable compound (original data not available)
Result : Other: not easily biodegradable
Source : Synthetic Chemicals Ltd. Wolverhampton
Test condition : Japanese MITI I test
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : aerobic
Inoculum :
Concentration : 100 mg/l related to Test substance
related to
Deg. product :
Method : other
Year : 2000
GLP : no data
Test substance :
Deg. products : Sulphate

Remark : This study investigated the aerobic biodegradation of sulfolane by two mixed microbial enrichments cultures and by three bacterial isolates. Sulfolane served as the sole carbon, sulphur and energy source for these cultures.
Result : In the two mixed cultures, 60% and 80% of the sulfolane carbon was recovered as carbon dioxide, whereas in cultures of the three isolates only 40-42% of the substrate carbon was recovered as carbon dioxide. In the mixed cultures, 81% and 97% of the sulfolane sulphur was converted to sulphate, and in the pure isolates, 55-90% of the substrate sulphur was converted to sulphate. Thus, the mixed cultures were capable of greater mineralisation than the pure isolates.
Test substance : other TS: aquifer materials from plumes contaminated with sulfur
Reliability : (2) valid with restrictions

Study reported in peer reviewed literature
Flag : Critical study for SIDS endpoint
23.06.2004 (14)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method : other: APHA No 219
Year : 1971
Concentration : 200 mg/l related to Test substance
BOD5 : 0 mg/l
GLP :
COD
Method : other: ASTM D 1252-67
Year : 1974
COD : 1.75 mg/g substance
GLP :
RATIO BOD5 / COD
BOD5/COD : 0

Remark : Th0D=1.73 g o/g substance.
Source : Synthetic Chemicals Ltd. Wolverhampton
Test condition : 10ml volume of effluent from a biological sanitary waste treatment plant (not adapted). The inoculum was checked with the reference chemical glucose and glutamic acid
Reliability : (4) not assignable
Seconday literature
17.06.2004 (16)

BOD5
Method : other: APHA Standard Method No. 219
Year : 1979
Concentration : related to
BOD5 : mg/l
GLP : no data
COD
Method : other: ASTM D 1252-67
Year : 1979
COD : 175 mg/g substance
GLP : no data

Result : BOD5 = 0.00 g/g
Reliability : (2) valid with restrictions
Study reported in peer reviewed literature
16.06.2004 (4)

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration :
Elimination : no data
Method : other: Japanese MITI reports
Year : 1977
GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Method : MITI bioaccumulation studies conducted in accordance with generally accepted principles.

Remark : Analytical conditions
Gas chromatograph: JGC-20 KFP, JOEL
Detector: Flame ionisation detector
Filler: PEG-20M 10%//Chromosorb W DMCS-AW 80-100 Mesh
Column: Glass, 2 mm ø x 2m
Column temperature: 180 degree C
Carrier gas: N2

Result : Bioconcentration factors:
Level 1 area: 1.3 (nominal concentration = 2.5 mg/L)
Level 2 area: 13 (nominal concentration = 25 mg/L)

Test condition : TEST ORGANISMS:
Weight: Approximately 26 g
Lengh: Approximately 11 cm
Pretreatment: The test fish were dipped in 10 ppm chlorotetracycline solution in a static state for 24 hours. They were then acclimated at 25°C for 12 days prior to the test.

TEST SYSTEM:
Concentration: 0.25, 2.5 mg/L
Test vessel: 100 L glass aquarium
Water flow rate: 579 L/day
Test temperature: 25±2 °C

The stock solution (10,000 mg/L) for exposure was prepared by dissolving the test substance in water. the exposure was conducted under flow-through conditions.

Reliability : (1) valid without restriction
The data are approved by the Japanese government

Flag : Critical study for SIDS endpoint

23.06.2004 (22)

Species :
Exposure period :
Concentration :
Elimination :
Method : other: calculation using BCF v2.15
Year : 2004
GLP :
Test substance : Name: Sulfolane (CAS No. 126-33-0)
Result : BCF Program (v2.15) Results:
==========
SMILES : O=S(=O)(CCC1)C1
CHEM : Thiophene, tetrahydro-, 1,1-dioxide
MOL FOR: C4 H8 O2 S1
MOL WT : 120.17
---------- Bcfwin v2.15 ----------
Log Kow (estimated) : -0.24
Log Kow (experimental): -0.77
Log Kow used by BCF estimates: -0.77

Equation Used to Make BCF estimate:
Log BCF = 0.50

Correction(s): Value
Correction Factors Not Used for Log Kow < 1

Estimated Log BCF = 0.500 (BCF = 3.162)

Reliability : (2) valid with restrictions
Estimated using EPIWIN v3.12

07.01.2005 (50)

3.8 ADDITIONAL REMARKS

Remark : Sulfolane affects organoleptic smell and taste of water at 0.58 and 0.69 mg/l, repectively. Sulfolane is not classified as a bioaccumulator or a tainter
Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	>= 100
LC50	:	> 100
LC100	:	> 100
Limit test	:	yes
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1999
GLP	:	yes
Test substance	:	

Method :

METHOD FOLLOWED: OECD guideline 203

DEVIATIONS FROM GUIDELINE: No

STATISTICAL METHODS:
Not applicable

METHOD OF CALCULATION:
Test substance concentration calculated using time-weighted means

ANALYTICAL METHODS:
Gas Chromatography:
Instrument: HP 5890 Series II
Detector: Flame ionisation detector
Auto injector: HP 7673 Injector
Column: 11 m x 0.32 m, made of fused silica
Liquid phase: HP-5, membrane thickness 0.25 µm
Column temperature: 70°C (1 min) rising to 250 °C (4 min)
Heating rate: 15°C/min
Inlet temperature: 320°C
Detector temperature: 320°C
Carrier gas: Helium
Split ratio: 8:1
Hydrogen: 1.4 kg/cm2
air: 2.4 kg/cm2
Injection volume: 1µL
Sensitivity:
Detector: Range 2 1 V/FS
Recorder: ATTEN 2(6)

Result : LC50 > 100 mg/L

OBSERVATIONS:

There were no mortalities during the study in either the control or test fish (Table 2).

There were no signs of toxicity in either the control or test fish during the study.

Test condition : TEST ORGANISMS:
Size: Length 1.80 - 2.0 cm, Weight: 0.083 - 0.14 g (n=10)

Age: No data
Pretreatment: The test fish were acclimatised for 12 days or more under the same conditions (water quality, temperature, etc) as the test conditions.
Supplier: Nakajima Pisciculture (Kumamoto Prefecture, Japan)

DILUTION WATER:
Source: Dechlorinated tap water supplied by Kurume City
Chemistry: Hardness 52.0 mg/L ($CaCO_3$), pH 7.5

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: A 10,000 mg/L stock test solution was prepared by dissolution of the required amount of test material in dilution water. Test solutions were prepared by mixing the required amount of stock test solution with dilution water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The measured test material concentrations were 97.8 and 103% of the nominal concentration at the initiation and completion of the exposure, respectively. Values were based on the nominal concentrations.

REFERENCE SUBSTANCE: Copper sulfate (II) pentahydrate (extra pure grade, Wako Pure Chemical Industries, Ltd.) LC50 (96 hr) = 1.22 mg/L.

TEST SYSTEM:
Concentrations: Control (dilution water), 100 mg/L
Renewal of test solution: Total amount of test water was replaced after 48 hours
Exposure vessel type: 3.0L glass container (diameter 16 cm, depth 17 cm)
Number of replicates, fish per replicate: 2 test vessels per concentration, 5 fish per vessel.
Test temperature: 24.2 - 24.8 °C
Dissolved oxygen: 6.9 - 8.3 mg/L
pH: 7.2 - 7.3
Intensity of irradiation: Room light
Photoperiod: 16 hours light/8 hours darkness
Feeding: No
Aeration: No

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 0 and 48 hour time periods.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 95.8%
Lot No.: PAH5988
Supplier: Wako Pure Chemical Industries Ltd.

Table 1: Concentration of sulfolane in test solutions

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percentage of nominal)		
	0-hour[(a)]	48-hour[(b)]	Mean[(c)]
Control	n.d.	n.d.	n.d.
100	97.8 (97.8)	103 (103)	100 (100)

n.d.: < 10.0 mg/L
fresh solutions
expired solutions
Time weighted means

Table 2: Mortality of orange killifish (Oryzias latipes) exposed to sulfolane

Nominal concentration (mg/L)	Cumulative number of dead fish (Percent mortality)			
	0-hour	48-hour	72-hour	96-hour
Control	0 (0)	0 (0)	0 (0)	0 (0)
100	0 (0)	0 (0)	0 (0)	0 (0)

Reliability : (1) valid without restriction
The data are approved by the Japanese government
Flag : Critical study for SIDS endpoint
05.05.2004 (43)

Type : semistatic
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC0 : 350
LC50 : > 1000
Limit test : no
Analytical monitoring : no
Method : other: Shell Research Ltd method
Year : 1982
GLP : no data
Test substance :
Test condition : TEST ORGANISMS:
Size: Length 3.3 – 5.3 cm, mean weight 0.8g (n=10)
Age: No data
Pretreatment: The test fish were acclimated to the test conditions for not less than 10 days prior to use.
Supplier: Itchen Valley Trout Farm, Alresford, Hampshire, UK

DILUTION WATER:
Source: Filtered dechlorinated mains water

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: stock solutions prepared by dissolving the chemical in distilled water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
No information

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: Control (dilution water), 100, 200, 350, 600, 1000 mg/L
Renewal of test solution: Total amount of test water was replaced at 24 hour intervals
Exposure vessel type: Glass aquaria containing 10 L of water.
Number of replicates, fish per replicate: 1 test vessel per concentration, 10 fish per vessel.
Test temperature: 15±1 °C
Dissolved oxygen: 9.8±0.3 mg/L
pH: 8.2±0.4
Intensity of irradiation: No information
Photoperiod: No information
Feeding: No
Aeration: Gentle aeration to maintain the concentration of dissolved oxygen.

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: None.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 97%
Impurities: Water (3%)
Lot No.: 696972
Supplier: Shell Chemicals UK

Results : See Table 1 below.
LC50 (96h) > 1000 mg/L.
Only 4 of 10 fish exposed to 1000 mg/L, the maximum concentration tested, had died after 96 hour exposure.

Table 1: Mortality of *S. Gairdneri* exposed to a range of concentrations of sulfolane

Concentration of sulfolane (mg/L)	No. of fish	Cumulative mortality			
		24h	48h	72h	96h
0 (control)	10	0	0	0	0
100	10	0	0	0	0
200	10	0	0	0	0
350	10	0	0	0	0
600	10	0	0	1	1
1000	10	3	4	4	4

Reliability : (2) valid with restrictions
No monitoring of test substance concentration

05.05.2004 (40)

Type : static
Species : Carassius auratus (Fish, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
LC50 : = 4800

Method	:	other: APHA, for the static-tank acute toxicity tests
Year	:	1971
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The article also gives a 24h-LC50 of 140 mg/l. This value is probably a mistake from the author. The original shell report AMGR.0095.3 gives only the LC50 value of 4800 mg/l.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test condition	:	Life-stage: 6.2 +/- 0.7 cm, 3.3 +/- g; D.O.>= 4 mg/l, pH 6-8, 20 degree oC, aerated
Reliability	:	(4) not assignable Secondary Literature

17.06.2004 (16)

Type	:	
Species	:	Gambusia affinis (Fish, fresh water)
Exposure period	:	90 hour(s)
Unit	:	mg/l
LC50	:	= 1930
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

Type	:	
Species	:	other: Stickleback
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 1760
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	171
EC50	:	852
Analytical monitoring	:	yes

Method : OECD Guide-line 202
Year : 1999
GLP : yes
Test substance :

Method : METHOD FOLLOWED: OECD guideline 202

DEVIATIONS FROM GUIDELINE: No

STATISTICAL METHODS:
EC50 calculated by the Probit method and recorded with the 95% confidence limit.

METHOD OF CALCULATION:
The percentage swimming inhibition in each test concentration medium was calculated from the number of Daphnia inhibited from swimming and the number of Daphnia used in the test (20). Calculation of the results was based on the nominal concentrations of the test material.

ANALYTICAL METHODS:
Gas Chromatography:
Instrument: HP 5890 Series II
Detector: Flame ionisation detector
Auto injector: HP 7673 Injector
Column: 18 m x 0.25 m, made of fused silica
Liquid phase: HP-5MS, membrane thickness 0.25 µm
Column temperature: 100°C (1 min) rising to 250 °C (4 min)
Heating rate: 15°C/min
Inlet temperature: 320°C
Detector temperature: 320°C
Split ratio: 19:1
Carrier gas: Helium
Total flow rate: 20.1 mL/min
Hydrogen: 1.4 kg/cm2
Air: 2.4 kg/cm2
Injection volume: 1µL
Sensitivity:
Detector: Range 2 1 V/FS
Recorder: ATTEN 2(5)

Result : EC50 (48 hr) = 852 mg/L (95% confidence limits = 695 - 1,190 mg/L)

The measured test material concentrations were 95.0 to 102% and 92.2 to 96.2% of the nominal concentrations at the initiation and completion of the exposure. Values were based on the nominal concentrations.

OBSERVATIONS:
none

Test condition : TEST ORGANISMS:
Age: < 24 hours old
Supplier: Sheffield University, UK

DILUTION WATER:
Source: Dechlorinated tap water supplied by Kurume City
Chemistry: Hardness 52.0 mg/L ($CaCO_3$), pH 7.5

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: A 10,000 mg/L stock test solution was prepared by dissolution of the required amount of test material in dilution water. Test solutions

were prepared by mixing the required amount of stock test solution with dilution water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The measured test material concentrations were 95.0 to 102% and 92.2 to 96.2% of the nominal concentrations at the initiation and completion of the exposure. Values were based on the nominal concentrations.

TEST SYSTEM:
Concentrations: Control (dilution water), 95.3, 171, 309, 556, 1000 mg/L
Renewal of test solution: No
Exposure vessel type: Petri dish (diameter 8.5 cm, depth 5.7 cm)
Number of replicates: 4
Individuals per replicate: 5
Test temperature: 20.2 - 20.5 °C
Dissolved oxygen: 8.7 - 8.8 mg/L
pH: 7.8 - 7.9
Intensity of irradiation: Room light
Photoperiod; 16 hours light/8 hours darkness
Feeding: No

TEST PARAMETER: Inhibition of swimming

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 0 and 48 hour time periods.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 95.8%
Lot No.: PAH5988
Supplier: Wako Pure Chemical Industries Ltd.

Table 1: Concentration of sulfolane in test solutions

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percentage of nominal)		
	0-hour[(a)]	48-hour[(b)]	Mean[(c)]
Control	n.d.	n.d.	n.d.
95.3	94.3 (99.0)	91.6 (96.2)	93.0 (97.6)
171	164 (95.6)	161 (93.9)	162 (94.8)
309	315 (102)	285 (92.2)	300 (97.0)
556	528 (95.0)	531 (95.4)	529 (95.2)
1000	1010 (101)	936 (93.6)	972 (97.2)

n.d.: < 10.0 mg/L
(a) fresh solutions
expired solutions
geometric means

Table 2: Immobility of Daphnia magna exposed to sulfolane

Nominal concentration (mg/L)	Cumulative number of immobilised daphnia (Percent immobilisation)	
	24-hour	48-hour
Control	0 (0)	0 (0)
95.3	0 (0)	0 (0)
171	0 (0)	0 (0)
309	0 (0)	1 (5)
556	3 (15)	3 (15)
1000	12 (60)	13 (65)

The values include dead Daphnia

Reliability : (1) valid without restriction
The data are approved by the Japanese government
Flag : Critical study for SIDS endpoint
05.05.2004 (42)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : 20
EC50 : 95
EC100 : 1000
Analytical monitoring : no
Method : other: Shell Research Ltd method
Year : 1982
GLP : no data
Test substance :

Test condition : TEST ORGANISMS:
Age: < 24 hours old
Supplier: In-house culture derived from a strain obtained from I.R.Ch.A., France

DILUTION WATER:
Source: Filtered dechlorinated mains water

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: Stock solutions prepared by dissolving the chemical in distilled water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
No information

TEST SYSTEM:
Concentrations: Control (dilution water), 10, 20, 50, 100, 200, 500, 1000 mg/L
Renewal of test solution: No
Exposure vessel type: 150 mL Petri dish
Number of replicates: 3
Individuals per replicate: 10
Test temperature: 20±1 °C
Dissolved oxygen: 9.2±0.3 mg/L
pH: 8.2±0.2
Intensity of irradiation: No information
Photoperiod; No information
Feeding: No

TEST PARAMETER: Inhibition of swimming

MONITORING OF TEST SUBSTANCE CONCENTRATION: None

Result : See Table 1 below
EC50 (24h) = 160 mg/L (95% confidence interval = 120 – 220 mg/L)
EC50 (48h) = 95 mg/L (95% confidence interval = 73 – 120 mg/L)

Table 1: Immobilisation of *D. Magna* exposed to a range of concentrations of sulfolane

Concentration of sulfolane (mg/L)	No. of *D. magna*	Cumulative total immobilised	
		24h	48h
0 (control)	10	0	0
	10	0	0
	10	0	0
10	10	1	0
	10	0	0
	10	0	0
20	10	0	0
	10	0	0
	10	0	0
50	10	1	4
	10	4	5
	10	2	4
100	10	3	4
	10	4	5

	10	5	6
200	10	6	7
	10	4	5
	10	6	8
500	10	6	9
	10	9	10
	10	9	10
1000	10	9	10
	10	8	10
	10	10	10

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 97%
Impurities: Water 3%
Lot No.: 696972
Supplier: Shell Chemicals UK

Reliability : (2) valid with restrictions
No monitoring of test substance concentration

05.05.2004 (40)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 40
Analytical monitoring : no data
Method :
Year :
GLP : yes
Test substance : other TS: Sulfolane aqueous

Remark : EC50 expressed as mg a.i./l
95% fiducial interval: 32-48 mg/l.
Source : Synthetic Chemicals Ltd. Wolverhampton
Test condition : Life-stage: < 24 h old; static; reconstituted fresh water, pH 7.8-8.2, hardness 168-178 mg/l CaCO3, D.O 8.6-9.4 mg/l, 18-22 degree c.
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

Type :
Species : other aquatic mollusc: Crassostrea gigas
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = 460
Analytical monitoring : no data
Method :
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Effect: susceptibility; embryo larval test
95% confidence interval: 420-500 mg/l
Source : Synthetic Chemicals Ltd. Wolverhampton
Test condition : Life-stage: embryo; static; artificial sea water (Tropic Marine 34 prom.), pH 7.9; D.O. 6.8-7.1 mg/l. 25 degree °C.
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

Type	:	
Species	:	other: aquatic crustacea, Acartia tonsa
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 52
Analytical monitoring	:	no
Method	:	
Year	:	
GLP	:	yes
Test substance	:	other TS: Sulfolane aqeous
Remark	:	EC50 expressed as mg a.i./l 95% fiducial interval; 34-80 mg/l
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test condition	:	Life-stage: adult, semi-static; full strength artificial sea water (Tropic Marin 34 prom, in distilled water), pH 7.8-8.2, D.O. 6.4-8.7 mg/l. 18-22 degree C.
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	Biomass and growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
NOECb	:	171
EbC50	:	500
ErC50	:	> 1000
NOECr (24-48 hr)	:	309
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1999
GLP	:	yes
Test substance	:	
Method	:	METHOD FOLLOWED: OECD guideline 201 DEVIATIONS FROM GUIDELINE: No STATISTICAL METHODS: NOEC: The specific values of the area under the growth curve and the growth rate were analysed for significance using one way analysis of variance and Dunnett's multiple comparison test. EC50: Regression method METHOD OF CALCULATION: Calculation of the results was based on the nominal concentrations of the test material. ANALYTICAL METHODS: Gas Chromatography: Instrument: HP 5890 Series II Detector: Flame ionisation detector

Auto injector: HP 7673 Injector
Column: 11 m x 0.32 m, made of fused silica
Liquid phase: HP-5, membrane thickness 0.25 µm
Column temperature: 70°C (1 min) rising to 250 °C (2 min)
Heating rate: 15°C/min
Inlet temperature: 320°C
Detector temperature: 320°C
Split ratio: 8:1
Carrier gas: Helium
Hydrogen: 1.4 kg/cm2
Air: 2.4 kg/cm2
Injection volume: 1µL
Sensitivity:
Detector: Range 2(1)1 V/FS
Recorder: ATTEN 2(6)

Result : EXPOSED:
Effect data/Element values:

Growth rate:

ErC50(0-72h) > 1000mg/L
ErC50 (24-48h) > 1000 mg/L
ErC50 (24-72h) > 1000 mg/L
NOECr (24-48h) = 309 mg/L
NOECr (24-72h) = 556 mg/L

Biomass:

EbC50 (0-72h) = 500 mg/L (95% C.I. 416 - 601 mg/L)
NOECb (0-72h) = 171 mg/L

It is considered that the results based on growth rate are more reliable than those for biomass, as growth rate has the more scientific basis.

Cell density data:
Growth curves: The cell density in the control medium increaesd 200-fold or more by the completion of exposure. This indicated normal growth of the algae under the conditions of this study. The algal growth in each test medium in comparison with that in the control medium was as follows: In the 1000, 556, and 309 mg/L media, algal growth was inhibited and the growth level was lower, but the growth process was similar to that in the control medium. In the 171 and 95.3 mg/L media, the algal growth was almost the same as that in the control medium.

CONTROL:
Number/percentage showing adverse effects: None
Nature of adverse effects: Not applicable

Test condition : TEST ORGANISMS:
Strain: ATCC22662
Supplier: American Type Culture Collection, Rockville, MD, USA
Preculture: 3 days before initiation of exposure under the same conditions used in the test.

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Medium: The medium prescribed in the OECD Chemicals Test Guidelines was used both in the preculture and the study.
Vehicle/solvent and concentration: None used
Preparation: A 10,000 mg/L stock test solution was prepared by addition of the medium to a required amount of test material and filtration sterilised

with a 0.45µm membrane filter. Test solutions were prepared by mixing a required amount of stock test solution with the medium for each concentration.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The measured test material concentrations were 97.6 to 102% and 91.6 to 98.7% of the nominal concntrations at the initiation and completion of the exposure, respectively. Values were based on the nominal concentrations.

REFERENCE SUBSTANCE: Potassium dichromate (reagent extra pure grade, Wako Pure Chemical Industries, Ltd.). EbC50 (72 hr) = 0.295 mg/L.

TEST SYSTEM:
Test type: Static, Shaking culture (100 rpm)
Concentrations: Control, 95.3, 171, 309, 556, 1000 mg/L
Renewal of test solution: No
Exposure vessel type: 500 mL glass Erlenmeyer flask fitted with a breathable silicone plug.
Number of replicates: 3 replicates per test concentration.
Initial cell density: 1E+04 cells/mL
Volume of test solution: 100 mL/vessel
Test temperature: 22.5 - 24.9°C
pH: Initial = 7.9 - 8.0, Final 8.6 - 9.9
Adjustment of pH: None
Intensity of irradiation: 4000 - 5000 lux
Photoperiod: continuous light

TEST PARAMETER: Biomass and growth rate

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 0 and 72 hour time periods.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 95.8%
Lot No.: PAH5988
Supplier: Wako Pure Chemical Industries Ltd.

Table 1: Concentration of sulfolane in test solutions

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percentage of nominal)		
	0-hour[(a)]	72-hour[(b)]	Mean[(c)]
Control	n.d.	n.d.	n.d.
95.3	97.5 (102)	93.9 (98.5)	95.7 (100)
171	171 (99.9)	165 (96.5)	168 (98.2)
309	303 (98.0)	305 (98.7)	304 (98.4)
556	565 (102)	537 (96.5)	551 (99.0)
1000	976 (97.6)	916 (91.6)	946 (94.6)

n.d.: < 10.0 mg/L
(a) fresh solutions
(b) expired solutions
(c) geometric means

Table 2: Cell density of Selenastrum capricornutum during 72-hour exposure to sulfolane

Nominal Concentration mg/L	Vessel No.	Cell Density (x10^4 cells/mL)			
		0	24	48	72
Control	1	1.0	6.6	48.3	208.6
	2	1.0	5.5	47.2	199.9
	3	1.0	6.7	57.0	242.1
	Average	1.0	6.3	50.8	216.9
	SD	0.0	0.6	5.4	22.3
95.3	1	1.0	6.4	49.4	221.4
	2	1.0	4.9	47.5	223.5
	3	1.0	6.1	48.8	177.8
	Average	1.0	5.8	48.5	207.6
	SD	0.0	0.8	1.0	25.8
171	1	1.0	5.5	39.0	163.9
	2	1.0	4.9	39.7	187.0
	3	1.0	5.4	41.2	183.8
	Average	1.0	5.3	40.0	178.2
	SD	0.0	0.3	1.1	12.5
309	1	1.0	5.5	42.5	186.5
	2	1.0	5.5	30.6	103.1
	3	1.0	5.6	34.3	147.9
	Average	1.0	5.5	35.8	145.8
	SD	0.0	0.1	6.1	41.8
556	1	1.0	5.0	16.3	90.1
	2	1.0	4.3	21.0	102.5
	3	1.0	5.4	25.1	111.5
	Average	1.0	4.9	20.8	101.4
	SD	0.0	0.5	4.4	10.7
1000	1	1.0	4.5	20.5	82.6
	2	1.0	5.1	13.9	46.9
	3	1.0	4.4	13.1	45.4
	Average	1.0	4.7	15.8	58.3
	SD	0.0	0.3	4.0	21.1

Table 3: Growth Inhibition of Selenastrum capricornutum during 72-hour exposure to sulfolane

Nominal Conc. (mg/L)	Vessel No.	Area (x10^4)	Inhibition (%)	Rate	Inhibition (%)	Rate	Inhibition (%)
		A(0-72h)	I_A(0-72h)	μ(24-48h)	Iμ(24-48h)	μ(24-72h)	Iμ(24-72h)
Control	1	3760	-	0.0831	-	0.072	-
	2	3600	-	0.0893	-	0.0747	-
	3	4370	-	0.0894	-	0.0748	-
	Average	3910	-	0.0873	-	0.0739	-
95.3	1	3940	-0.608	0.0853	2.22	0.0739	-0.0610
	2	3880	0.849	0.0944	-8.19	0.0795	-7.61
	3	3390	13.3	0.0866	0.752	0.0703	4.90
	Average	3740	4.52	0.0888	-1.74	0.0746	-0.923
171	1	2970	24.0	0.0819	6.16	0.0709	4.08
	2	3260	16.8	0.0870	0.343	0.0758	-2.55
	3	3260	16.6	0.0846	3.12	0.0734	0.595
	Average	3170	19.1	0.0845	3.21	0.0734	0.708

309	1	3330	14.9	0.0856	1.94	0.0736	0.364
	2	2040	47.8	0.0715	18.1	0.0611	17.3
	3	3670	31.7	0.0757	13.3	0.0683	7.59
	Average	2680	31.4	0.0776	11.1	0.0676	8.43
556	1	1530	60.8	0.0493	43.5	0.0603	18.3
	2	1780	54.6	0.0657	24.7	0.0659	10.8
	3	2010	48.6	0.0643	26.3	0.0632	14.5
	Average	1770	54.7	0.0598	31.5	0.0631	14.5
1000	1	1530	60.8	0.0628	28.1	0.0604	18.2
	2	960	75.5	0.0421	51.8	0.0463	37.3
	3	906	76.8	0.0451	48.3	0.0484	34.4
	Average	1130	71.1	0.0500	42.7	0.0517	30.0

Reliability : (1) valid without restriction
The data are approved by the Japanese government
Flag : Critical study for SIDS endpoint
05.05.2004 (44)

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : 1000
EC50 : > 1000
Limit test : no
Analytical monitoring : no
Method : other: Shell Research Ltd method
Year : 1982
GLP : no data
Test substance :
Test condition : TEST ORGANISMS:
Strain: ATCC22662
Supplier: In-house axenic culture derived from a strain obtained from American Type Culture Collection, Rockville, MD, USA
Preculture: No information

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Medium: The medium was based on Miller WE and Green JC (1978). The *Selenastrum capricornutum* (Prinz) algal assay bottle test.
Vehicle/solvent and concentration: None used
Preparation: Stock solutions prepared by dissolving the chemical in distilled water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS: No information.

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Test type: Static, Shaking culture (100 rpm)
Concentrations: 0 (control), 100, 140, 160, 190, 230, 330, 400, 480, 580, 700, 830, 1000 mg/L
Renewal of test solution: No
Exposure vessel type: Erlenmeyer flask
Number of replicates: 6 control flasks, one flask per test concentration.
Initial cell density: 5E+03 cells/mL
Volume of test solution: 50 mL/vessel
Test temperature: 24±1°C
pH: No information
Adjustment of pH: None
Intensity of irradiation: No information

Photoperiod: continuous light

TEST PARAMETER: Growth rate

MONITORING OF TEST SUBSTANCE CONCENTRATION: None

Results : See Table 1 below

None of the concentrations tested caused a 50% reduction in the growth rate over the period day 2 to day 4 compared to the growth rate of the controls. The EC50 value based on growth rate for the period day 2 to day 4 was therefore greater than 1000 mg/L, the maximum concentration tested.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 97%
Impurities: Water (3%)
Lot No.: 696972
Supplier: Shell Chemicals UK

Reliability : (2) valid with restrictions
No monitoring of test substance concentration

05.05.2004 (40)

Table 1: Growth of *S. Capricornutum* cultures exposed to a range of concentrations of sulfolane

Concentration of sulfolane (mg/L)	Cell concentration (cells/mL x 10^{-6})		Mean relative growth rate	
	Day 2	Day 4	Day 2 – Day 4	As % of mean control relative growth rate
0 (control)	0.04	1.2	1.7	
0	0.04	1.6	1.8	
0	0.04	1.5	1.8	
0	0.04	1.6	1.8	
0	0.04	1.3	1.7	
0	0.04	1.5	1.8	
100	0.04	1.7	1.8	104
140	0.04	1.5	1.8	105
160	0.04	1.7	1.9	105
190	0.04	1.4	1.8	100
230	0.04	1.6	1.8	99
330	0.04	1.9	1.9	107
400	0.04	1.4	1.7	98
480	0.04	1.7	1.9	105
580	0.03	1.8	2.0	111
700	0.04	1.7	1.8	102
830	0.03	1.6	2.0	111
1000	0.04	1.8	1.8	104

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)

Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	25
LCEC	:	50
EC50	:	> 100
LC50	:	> 100
Analytical monitoring	:	yes
Method	:	OECD Guide-line 211
Year	:	1999
GLP	:	yes
Test substance	:	

Method :

METHOD FOLLOWED: OECD guideline 211

DEVIATIONS FROM GUIDELINE: No

STATISTICAL METHODS:
Parental mortality: Kruskal-Wallis' analysis of variance by ranks and Dunnett's non-parametric multiple comparison test.
First delivery day: Kruskal-Wallis' analysis of variance by ranks.
Mean total fertility: One way analysis of variance and Scheffe's multiple comparison test.
NOEC and LOEC (reproduction): One way analysis of variance and Scheffe's multiple comparison test.

METHOD OF CALCULATION:
Calculation of the results was based on the nominal concentrations of the test material.

ANALYTICAL METHODS:
Gas Chromatography:
Instrument: HP 5890 Series II
Detector: Flame ionisation detector
Auto injector: HP 7673 Injector
Column: 11 m x 0.32 m, made of fused silica
Liquid phase: HP-5, membrane thickness 0.25 µm
Column temperature: 70°C (1 min) rising to 250 °C (2 min)
Heating rate: 15°C/min
Inlet temperature: 320°C
Detector temperature: 320°C
Split ratio: 8:1
Carrier gas: Helium
Hydrogen: 1.4 kg/cm2
Air: 2.4 kg/cm2
Injection volume: 1µL
Sensitivity:
Detector: Range 2(1)1 V/FS
Recorder: ATTEN 2(6)

Result : Number of mortalities and death rate: The cumulative mortality of parent water fleas in the control medium was 0% at the completion of exposure, which was within the validity range of 20% or less.

There were no deaths in the 25.0 mg/L medium. The cumulative mortality of the 50.0 and 100 mg/L media were each 40% at the completion of exposure, but no statistically significant differences were noted compared with the control medium.

Day of first birth: The first delivery day was Day 8 for all individual water fleas in the control and the treatment group.

Mean total fertility: The mean total fertility per parent water flea in the control medium was 178, which was over the minimum limit of 60.

The mean total fertility per parent water flea in the 25.0 and 50.0 mg/L media were 155 and 160, respectively, showing no significant difference compared with the control medium. The mean total fertility per parent water flea in the 100 mg/L medium was 96.5, which was smaller than that in the control and showed a statistically significant difference.

Size and appearance of parent water fleas: No growth inhibition was observed in each concentration medium compared with the control medium throughout the exposure period. Lightning of body colour and adherence of chlorella to tentacles were noted in all test media. Several water fleas from the 50.0 mg/L medium also showed swimming inhibition and hypoactivity

Production of diapause eggs, etc: No diapause eggs were produced in any of the test media throughout the exposure period. Death of offsprings was noted in the control and all test concentration media and the number of deceased ofsprings in the 25.0 and 50.0 mg/L media was slightly larger than in the control medium. Fallen eggs were noted in the control and all test concentration media, but the number was small.

Median Lethal Concentration (LC50) of parent water fleas:

LC50 (14 day) > 100 mg/L
LC50 (21 day) > 100 mg/L

Median reproduction inhibition concentration:

EC50 (14 days) > 100 mg/L
EC50 (21 days) > 100 mg/L

NOEC and LOEC affecting reproduction:

As a result of the significance test of the mean total fertility, the 25.0 and 50.0 mg/L media showed no significant difference, but the 100 mg/L medium showed a significantly smaller value than the control medium. In the 50 mg/L medium, however, the cumulative mortality of parent water fleas was 40% at the completion of exposure. It was therefore considered that NOEC (21 day, reproduction) = 25.0 mg/L and LOEC (21 day, reproduction) = 50 mg/L.

Test condition : TEST ORGANISMS:
Age: < 24 hours old
Supplier: Sheffield University, UK

DILUTION WATER:
Source: Dechlorinated tap water supplied by Kurume City
Chemistry: Hardness 52.0 mg/L ($CaCO_3$), pH 7.5

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent and concentration: None used
Preparation: A 1,000 mg/L stock test solution was prepared by dissolution of the required amount of test material in dilution water. Test solutions were prepared by mixing the required amount of stock test solution with dilution water.

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The measured test material concentrations were 95.9 to 106% of nominal at initiation and 96.6 to 103% of nominal prior to renewal. Values were based on the nominal concentrations.

REFERENCE SUBSTANCE: Potassium dichromate (extra pure grade, Wako Pure Chemical Industries, Ltd.) EiC50 (48 hr) = 0.135 mg/L.

TEST SYSTEM:
Concentrations: Control (dilution water), 25.0, 50.0, 100 mg/L
Renewal of test solution: Semistatic, renewal 3 times per week
Exposure vessel type: 100 mL glass beaker
Volume of test solution: 80 mL
Number of replicates: 10
Individuals per replicate: 1
Test temperature: 20.0 - 20.3 °C
Dissolved oxygen: 8.3 - 8.8 mg/L
pH: 7.5 - 8.6
Hardness: 43.0 - 49.0 mg CaCO3/L (measured)
Intensity of irradiation: Room light
Photoperiod; 16 hours light/8 hours darkness
Feeding: Chlorella vulgaris, 0.1-0.2 mg organic carbon/water flea/day

TEST PARAMETER: Number of offspring produced for 21 days.

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at initiation and prior to renewal.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 95.8%
Lot No.: PAH5988
Supplier: Wako Pure Chemical Industries Ltd.

Table 1: Measured concentrations of test substance during a 21-day exposure of *Daphnia magna* under the semi-static test conditions

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (Percent of Nominal)						Time-Weighted Mean during 18-days (mg/L)
	0 day new	2 day old	11 day new	14 day old	16 day new	18 day old	
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
25.0	24.9 (99.6)	24.3 (97.0)	24.6 (98.3)	24.2 (96.6)	25.6 (102)	24.2 (97.0)	24.6 (98.3)
50.0	50.8 (102)	49.1 (98.2)	47.9 (95.9)	51.2 (102)	51.8 (104)	48.8 (97.6)	49.9 (99.7)
100	99.1 (99.1)	101 (101)	97.7 (97.7)	103 (103)	106 (106)	96.7 (96.7)	101 (101)

new: freshly prepared test solution
old: Prior to renewal

Table 2: Cumulative Numbers of Dead Parental Daphnia

Nominal conc. (mg/L)	Days																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
25.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
50.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	4
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(10)	(10)	(20)	(40)
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	4
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(10)	(10)	(30)	(30)	(40)

The values in parentheses express mortality (%) of Daphnia

Table 3: Time (day) to First Brood Production

Nominal concn (mg/L)	Vessel No.										
	1	2	3	4	5	6	7	8	9	10	Mean
Control	8	8	8	8	8	8	8	8	8	8	8
25.0	8	8	8	8	8	8	8	8	8	8	8
50.0	8	8	8	8	8	8	8	8	8	8	8
100	8	8	8	8	8	8	8	8	8	8	8

Table 4: Mean cumulative Numbers of Juveniles Produced per Adult (ΣF1/P)

Nominal Conc. (mg/L)	Days																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	21.2	21.3	21.3	62.0	62.0	62.0	106	106	106	109	146	146	146	178
25.0	0	0	0	0	0	0	0	0	20.0	20.0	20.0	55.9	55.9	55.9	96.6	97.2	97.2	107	132	132	132	155
50.0	0	0	0	0	0	0	0	0	18.7	18.7	18.7	53.0	53.0	53.0	94.3	94.3	94.5	107	132	132	132	160
100	0	0	0	0	0	0	0	0	19.5	19.5	19.5	51.5	51.5	51.5	77.5	80.2	80.2	80.2	93.0	93.0	93.0	96.5

Table 5: Mean cumulative numbers of juveniles produced per adult in control and test vessels after 21 days

Nominal concn (mg/L)	Vessel No.												
	1	2	3	4	5	6	7	8	9	10	Mean	S.D.	Significant Difference
Control	184	190	185	187	169	186	183	170	202	128	178	20.1	
25.0	133	136	159	196	171	180	116	109	185	168	155	30.1	
50.0	-	113	186	170	176	-	172	-	141	-	160	27.4	
100	106	18	-	145	-	101	115	-	94	-	97	42.4	**

**: significantly different from control at $p < 0.01$

As a result of the significance test of the mean total fertility, the 25.0 and 50.0 mg/L media showed no significant difference, but the 100 mg/L medium showed a significantly smaller value than the control medium. In the 50 mg/L medium, however, the cumulative mortality of parent water fleas was 40% at the completion of exposure. It was therefore considered that NOEC (21 day, reproduction) = 25.0 mg/L and LOEC (21 day, reproduction) = 50 mg/L.

Reliability : (1) valid without restriction
The data are approved by the Japanese government
Flag : Critical study for SIDS endpoint
05.05.2004 (45)

Species	:	Mysidopsis bahia (Crustacea)
Endpoint	:	other: growth
Exposure period	:	7 day(s)
Unit	:	mg/l
NOEC	:	= 150
LCEC	:	> 150
Analytical monitoring	:	no
Method	:	other: EPA, 7 day Mysidopsis bahia static renewal, survival, growth and fecundity test
Year	:	1987
GLP	:	no data
Test substance	:	other TS: sulfolane -w
Remark	:	Marine Species Other toxicity data: 7d LC50 : 344 mg/l 7d LOEC survival: 300 mg/l 7d NOEC survival: 150 mg/l
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test substance	:	Sulfolane-W (97% 1,1-tetrahydrothiophenedioxide and 3% water)
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

Species	:	other aquatic crustacea: Ceriodaphnia dubia
Endpoint	:	reproduction rate
Exposure period	:	7 day(s)
Unit	:	mg/l
NOEC	:	< 375
LCEC	:	= 375
Analytical monitoring	:	no
Method	:	other: EPA, three brood, 6-8 day Ceriodaphnia dubia static renewal, survival and reproduction test
Year	:	1989
GLP	:	no data
Test substance	:	other TS: Sulfolane-W
Remark	:	Other toxicity data: 7d LC50: 2575 mg/l 7d LOEC survival: 3000 mg/l 7d NOEC survival: 1500 mg/l
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test substance	:	Sulfolane-W (97% 1.1-tetrahydrothiophenedioxide and 3% water)
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	*Lactuca sativa*
Endpoint	:	

Exposure period :
Unit :
NOEC :
LCEC :
Analytical monitoring :
Method :
Year : 2001
GLP :
Test substance : Sulfolane (CAS No. 126-33-0)

Method : This study, conducted on lettuce (*Lactuca sativa*) consisted of a five day seed germination/root elongation test using fine-textured soil (till). This is a widely used and accepted short term test for plants*.

*See, for example, ASTM (1990) Standard guide for conducting seedling emergence toxicity tests in soils and sediments from hazardous waste sites, Draft, Annual Book of ASTM Standards. Committee E-47 on Biological Effects and Environmental Fate (E47.11.01 Plant Toxicology).

Results : See Table 1
LOEC (root elongation) = 570 mg/kg
LOEC (seed germination) = 1200 mg/kg
NOEC (root elongation) = 290 mg/kg
NOEC (seed germination) = 570 mg/kg

Remark : Original reference: Komex International Ltd (1999) Shell Waterton Complex: Regional Sulfolane and Diisopropanolamine (DIPA) groundwater contamination situation, status to the end of 1998. Report prepared for Shell Canada Ltd.

Table 1: Toxicity of Sulfolane to Lettuce (*Lactuca sativa*)

Endpoint	Soil type	NOEC (mg/kg)	LOEC (mg/kg)	EC25 (mg/kg)	EC50 (mg/kg)
Root elongation	Till	290	570	1300	2200
Germination	Till	570	1200	1400	1800

Reliability : (2) valid with restrictions
Full experimental details not available from the online version of the document
17.06.2004 (18)

Species : *Lactuca sativa, Daucus Carota, Medicago sativa, Phleum pratense*
Endpoint :
Exposure period :
Unit :
NOEC :
LCEC :
Analytical monitoring :
Method :
Year : 2001
GLP :
Test substance : Sulfolane (CAS No. 126-33-0)

Method : In this study, conducted using an Environment Canada draft protocol*, four plant species (lettuce (*Lactuca sativa*), carrot (*Daucus carota*), alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*)) and four soils with differing texture, organic carbon content and cation exchange capacity (see Table 1) were examined.

The endpoints measured were emergence, biomass, root length, and shoot length.

*Environment Canada (1998) Development of Plant Toxicity Tests for Assessment of Contaminated Soils. Prepared for Method Development and Application Section, Environmental Technology Centre, Environment Canada; by Aquaterra Environmental, Orton, Ontario, November 1998.

Results : See Table 2a-d

For all four plant species, the most sensitive endpoint was root length elongation, with the lowest LOECs ranging from 440 mg/kg (alfalfa in till) to 940 mg/kg (lettuce emergence, lettuce root length, and carrot root length in till). The highest LOEC was > 23,700 mg/kg for alfalfa shoot length in loam. Plants were generally most sensitive to sulfolane in till and least sensitive in loam.

Table 1: Soil Characteristics

Soil	Sand (%)	Silt (%)	Clay (%)	Water holding capacity (mL/100g)	pH	Cation exchange capacity (meq/100g)	Organic carbon content (%)	Conductivity (µS/m)
Artificial soil	84	1	15	48	7.0	3.0	1.4	181
Sand	80	7	13	55	8.0	4.0	0.8	475
Loam	46	33	21	96	7.6	22	14	693
Till	54	27	19	64	7.7	10	0.8	148

Table 2a: Toxicity of Sulfolane to Lettuce (*Lactuca sativa*)

Endpoint	Soil type	NOEC (mg/kg)	LOEC (mg/kg)	EC25 (mg/kg)	EC50 (mg/kg)
Emergence	Artifiical	944	1890	1530	2690
	Loam	5400	10800	6650	9830
	Sand	911	1820	1030	1430
	Till	440	940	940	1410
Biomass	Artifiical	944	1890	462	1780
	Loam	10800	>10800	>10800	>10800
	Sand	1820	>1820	>1820	>1820
	Till	1880	>1880	>1880	>1880
Root length	Artificial	944	1890	1370	2470
	Loam	2700	5400	7000	9840
	Sand	455	911	526	1070
	Till	440	940	572	1260
Shoot length	Artificial	1890	3780	2520	>3780
	Loam	21600	>21600	>21600	>21600
	Sand	455	911	650	>1820
	Till	940	1880	1690	>1880
Minimum Toxicity Values for Lettuce		**290**	**570**	**462**	**1070**

Table 2b: Toxicity of Sulfolane to Carrot (*Daucus carota*)

Endpoint	Soil type	NOEC (mg/kg)	LOEC (mg/kg)	EC25 (mg/kg)	EC50 (mg/kg)
Emergence	Artifiical	3780	7550	4430	6340
	Loam	10800	21600	11600	19400
	Sand	1820	3640	2280	3430
	Till	1880	3760	3410	4830
Biomass	Artifiical	1890	3780	2560	>7550
	Loam	21600	>21600	>21600	>21600
	Sand	3640	>3640	2770	>3640
	Till	3760	>3760	>3760	>3760
Root length	Artificial	944	1890	1220	2390
	Loam	2700	5400	14100	17300
	Sand	455	911	512	1800
	Till	440	940	807	2390
Shoot length	Artificial	1890	3780	4040	6780
	Loam	10800	21600	11800	>21600
	Sand	1820	3640	2420	>3640
	Till	1880	3760	3070	>3760
Minimum Toxicity Values for Carrot		**440**	**911**	**512**	**1800**

Table 2c: Toxicity of Sulfolane to Alfalfa (*Medicago sativa*)

Endpoint	Soil type	NOEC (mg/kg)	LOEC (mg/kg)	EC25 (mg/kg)	EC50 (mg/kg)
Emergence	Artifiical	3780	7550	5760	8180
	Loam	23700	47300	29800	35900
	Sand	3640	7290	4320	5740
	Till	3760	7510	4180	5340
Biomass	Artifiical	944	1890	2210	>7550
	Loam	23700	>23700	>23700	>23700
	Sand	7290	>7290	>7290	>7290
	Till	3760	>3760	>3760	>3760
Root length	Artificial	944	1890	1810	3120
	Loam	5920	11800	8390	11100
	Sand	911	1820	931	1490
	Till	235	440	490	1530
Shoot length	Artificial	7550	>7550	>7550	>7550
	Loam	23700	>23700	>23700	>23700
	Sand	1820	3640	4200	6070
	Till	3760	>3760	>3760	>3760
Minimum Toxicity Values for Alfalfa		**235**	**440**	**490**	**1530**

Table 2d: Toxicity of Sulfolane to Timothy (*Phleum pratense*)

Endpoint	Soil type	NOEC (mg/kg)	LOEC (mg/kg)	EC25 (mg/kg)	EC50 (mg/kg)
Emergence	Artifiical	1890	3780	2990	4630
	Loam	10800	21600	14000	20000
	Sand	455	911	2320	3160
	Till	1880	3760	2150	3070
Biomass	Artifiical	7550	>7550	3260	6730
	Loam	675	1350	1050	2960
	Sand	228	455	384	>3640
	Till	3760	>3760	1430	2930
Root length	Artificial	472	944	1030	1990
	Loam	1350	2700	4050	9350
	Sand	455	911	562	911
	Till	n/a	n/a	n/a	n/a
Shoot length	Artificial	1890	3780	3310	5300
	Loam	5400	10800	13100	18400

	Sand	911	1820	1530	2560
	Till	940	1880	1820	3110
Minimum Toxicity Values for Timothy		**228**	**455**	**384**	**911**

Reliability : (2) valid with restrictions
Full experimental details not available from the online version of the document
17.06.2004 (18)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : rat
Number of animals
Males : 3
Females :
Doses
Males : 500, 1000 mg/kg
Females :
Vehicle : no data
Route of administration : i.v.
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour : CNS stimulation
Deg. product : not measured
Method : other: Andersen et al (1976)
Year : 1976
GLP : no data
Test substance :

Method : Experiments using rats:
Number of animals: 3/dose
Dose levels: 500, 1000 mg/kg
Dose route: i.v.

The amount of sulfolane excreted unchanged in the urine was measured daily for 7 days after administration.

Experiments using rabbit, dog and squirrel monkey (Saimiri sciurea):

In a limited series of experiments on individual animals, blood-sulfolane decay curves were obtained following intravenous injections of sulfolane. From these curves, the plasma half-life and volume of distribution were determined for the animals.

Method of analysis:

Sulfolane analysis was performed using gas-liquid chromatography. Blood sulfolane concentrations were assayed by direct injection of blood into the chromatograph equipped with disposable glass liners in the injection port which was heated to 300 deg C. Urine, diluted 2:1 with saturated sodium acetate solutions, were extracted with chloroform. The chloroform extract was evaporated, the residue dissolved in water, and the aqueous solution assayed for sulfolane.

Chromatographic conditions:
Column: 10% Carbowax 2000M on 60/80 Chromosorb-W (6 ft x 0.25 in)
Detector: FID
Injection temperature: 200 deg C
Column temperature: 190 deg C

Flame detector temperature: 255 deg C
Helium flow: 60 mL/min

Result : At doses of 500 or 1000 mg/kg (administered i.v.) large amounts of sulfolane were excreted unchanged in the urine and the proportion of a dose excreted unchanged by the rat increased as dosage increased (see table below).

The observation that the proportion of a dose recovered increased with dosage suggests that the metabolic pathway is saturable.

Excretion of unmetabolized sulfolane in urine after a single dose continued for several days, which is in accord with the plasma half-life of sulfolane of about 5 hr.

In the experiments on a dog, rabbit and squirrel monkey, sulfolane was rapidly distributed throughout the animals with volumes of distribution of near 1.0 l/kg, and was slowly removed from plasma with a half-life of 3.5 to 5 hr.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: no data
Lot No.: no data
Supplier: Shell Chemical Co.

Table 1: The cumulative percentage of an intravenous dose of sulfolane excreted unchanged in the urine by the rat

Dose (mg/kg)	% dose excreted in urine		
	Day		
	0-2	0-4	0-7
500	28±6	36±11	37±11
1000	50±1.2	67.2±2	-

Reliability : (2) valid with restrictions
Study reported in peer reviewed literature

12.05.2004 (2)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : rat
Number of animals
Males : 3
Females :
Doses
Males : 100mg/rat
Females :
Vehicle : water
Route of administration : i.p.
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product : yes
Method : other: Roberts et al (1960)
Year : 1961

GLP : no
Test substance :

Method : 35S-tetrahydrothiophene-1:1-dioxide was injected intraperitoneally into 3 male Wistar rats (100 mg/rat in water (2 mL) and the 24-hr urinary samples were examined separately.

Metabolites in the urine were separated using paper chromatography and autoradiographs performed.

Attempts were made to characterise the chemical properties of the major metabolite in order to elucidate its structure.

In a second experiment, 3 rabbits were each injected intraperitoneally with unlabelled tetrahydrothiophene-1:1-dioxide (1g) and 35S-tetrahydrothiophene-1:1-dioxide (100 mg) and the urine from each animal was collected, combined and extracted with chloroform. The residual extract was then identified.

Result : In the initial study performed using rats, one major metabolite was excreted in the 24-hr urine samples from the animals. This constituted 85% of the urinary radioactivity. Analysis of the metabolite from the follow-up rabbit study identified the metabolite as 3-hydroxy sulfolane.

Test substance : Name: 35S-tetrahydrothiophene-1:1-dioxide
Reliability : (2) valid with restrictions
Study reported in peer reviewed literature

12.05.2004 (36)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value :
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 70
Vehicle : water
Doses : 0, 892, 1204, 1626, 2191, 2963, 4000 mg/kg
Method : OECD Guide-line 401 "Acute Oral Toxicity"
Year : 1996
GLP : yes
Test substance :

Result : LD50 (males) = 2006 mg/kg
LD50 (females) = 2130 mg/kg

Mortality: See table below. All deaths occurred on the day of dosing.

Clinical Observations: Clinical signs of convulsion as well as decreased locomotor activity, ptosis, salivation, piloerection, chromodacryorrhea and perineal region soiling with urine were observed in the treated groups.

Bodyweight: Body weights of the treated animals were lower than those of the control group on the day after dosing.

Necropsy: Dead animals showed haemorrhagic black spots in

their glandular stomach mucosa.

Test condition : Age at study initiation: 5 weeks old for both sexes
Weight at study initiation: 122-138 g for males, 107 - 120 g for females
No. of animals/sex/group: 5
Mode of administration: gavage

Clinical observations performed and frequency: Each rat was weighed prior to treatment, on the day of treatment and 1, 3, 7 and 14 days post treatment. the rats were observed periodically during the 2 week post treatment period for signs of toxicity. All rats were submitted for a gross pathological examination.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 95%
Impurities: water (5%)
Lot No.: 0007
Supplier: Shin Nippon Rika

Table 1: Mortality of male and female rats after acute oral exposure to Sulfolane

Dose level (mg/kg)	No. Dead/No. Treated	
	Males	Females
0	0/5	0/5
892	0/5	0/5
1204	0/5	0/5
1626	0/5	0/5
2191	4/5	3/5
2963	5/5	5/5
4000	5/5	5/5

Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP

Flag : Critical study for SIDS endpoint

05.05.2004 (23)

Type : LD50
Value : 2473 mg/kg bw
Species : rat
Strain : other: Crl:CD.BR
Sex : male/female
Number of animals : 32
Vehicle : water
Doses : 1600, 2240 and 3136 mg/kg
Method : Directive 84/449/EEC, B.1 "Acute toxicity (oral)"
Year : 1993
GLP : yes
Test substance :

Result : The LD_{50} was 2489 mg/kg (males), 2324 mg/kg (females) and 2473 mg/kg (combined)

Mortality:
Deaths occurred from two hours after dosing until Day 2 among rats treated at the intermediate and high dose levels.

Clinical observations:
Fasciculation, tremor, twitching, splayed gait, hunched posture, piloerection, unkempt appearance and yellow staining of the anogenital fur. Convulsions and salivation developed among the rats dosed at 2240 and 3136 mg/kg. Isolated cases of hypersensitivity to stimuli, hyperactivity, lethargy, hypothermia, diarrhoea, lachrymation, pallor of the eyes and blood around the mouth were also observed. Onset of the principal clinical signs was generally apparent within four hours of dosing. Recovery of survivors, as judged by external appearance and behaviour, was advanced by Day 3, with one exception, was complete by Day 6.

All surviving rats had gained weight relative to their Day 1 bodyweights by the end of the first and second weeks of the 14 day observation period.

Necropsy:
Findings amongst the decedents were lung congestion, exaggerated lobular pattern or dark patches on the liver, darkening of the spleen or kidneys and abnormal contents (colourless liquid or gaseous) of the gastrointestinal tract, especially the stomach and small intestine. Rats killed at completion of the observation period showed no macroscopic changes other than a single case of hepatic pallor.

Test condition : Age at study initiation: 5-6 weeks old for both sexes
No. of animals/sex/group: 5
Mode of administration: gavage

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: 97.4%
Impurities: water (2.6%)
Lot No.: AIS00057; Tank T1413
Supplier: Shell Chemical Co.

Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP

05.05.2004 (12)

Type : LD50
Value : 2362.5 mg/kg bw
Species : rat
Strain : no data
Sex : male/female
Number of animals : 60
Vehicle : no data
Doses : Males: 1000, 1500, 2000, 3000, 5000 mg/kg. Females: 1000, 2000, 2500, 3000, 5000 mg/kg
Method : other: Hazleton Laboratories method
Year : 1983
GLP : no
Test substance :

Result : LD50 = 2739 (males)
LD50 = 2107.9 (females)
LD50 = 2362.5 (combined)

Clinical observations:
One male and five females dosed at 1000 mg/kg and four males dosed at 1500 mg/kg bodweight appeared normal from initiation to termination.

Clinical observations were noted in the remaining animals and included one or more of the following: depression, slight depression, rough coat, salivation, a hunched appearance, tremors, ataxia, urine stains, soft faeces, and red stains on the nose and/or eyes. All surviving rats that showed clinical signs appeared normal by Day 3 through to termination of

the study.

Weight gain:
All rats that survived to termination gained weight from initiation to termination. All rats that died lost weight except two which gained weight and two which maintained the same weight.

Gross pathology:
No observable gross pathology was noted in rats surviving to termination. Alterations of the stomach and/or intestines were the most common findings amongst animals that died. These alterations included compound like material, dark red material, reddish fluid or yellowish fluid in the stomach and/or intestines. Findings in the lung and liver were noted at the 5000 mg dose only.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Study reported in peer reviewed literature

05.05.2004 (33)

Type : LD50
Value : 2100 mg/kg bw
Species : rat
Strain : Wistar
Sex : male/female
Number of animals : 8
Vehicle : no data
Doses : no data
Method : other: Brown et al method
Year : 1966
GLP : no
Test substance :

Result : All animals that died did so witihn 24 hours and mostly in less than three hours. In each case death was preceded by convulsions and gasping for breath.

Necropsies on animals that died revealed no specific pathological lesion and it was believed that anoxia was the cause of death.

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: anhydrous
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

05.05.2004 (5)

Type : LD50
Value : 1.54 ml/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Method : other: Smyth et al (1962)
Year : 1969
GLP : no
Test substance :

Test condition : Number of animals: 5/dose

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient experimental detail
05.05.2004 (39)

Type : LD50
Value : 1846 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Method : other: Andersen et al method
Year : 1976
GLP : no
Test substance :

Method : Groups of 4-6 animals were dosed with various concentrations of sulfolane and the LD50 calculated from the mortality after one week by the method of Weil (1952). Animals were fasted overnight prior to determination of the oral LD50.
Result : Rats assumed a hunched, retreating posture with front limbs braced wide, tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions. In the post-ictal period rats were unresponsive to loud noises and pain, resisted changes in position with only a weak righting reflex and had a weak corneal response. These toxic signs were attributed to a CNS excitatory effect, following a period of post-ictal depression.
Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: no data
Lot No.: no data
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail
05.05.2004 (2)

Type : LD50
Value : 2500 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other: Filippova (1968)
Year : 1968
GLP : no data
Test substance :

Result : LD100 = 3500 mg/kg
Test substance : Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient experimental detail
05.05.2004 (11)

Type : LD50

Value : 1900 - 2500 mg/kg bw
Species : mouse
Strain : no data
Sex : male/female
Number of animals : 4
Vehicle : no data
Doses : no data
Method : other: Brown et al (1966)
Year : 1966
GLP : no
Test substance :

Result : All animals that died did so witihn 24 hours.

Convulsions and gasping for breath observed.
Reliability : (4) not assignable
Insufficient experimental detail
05.05.2004 (5)

Type : LD50
Value : 1600 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other:Filippova et al (1968)
Year : 1968
GLP : no
Test substance :

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient experimental detail
05.05.2004 (11)

Type : LD50
Value : 1815 mg/kg bw
Species : guinea pig
Strain : Hartley
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Method : other: Andersen et al (1976)
Year : 1976
GLP : no
Test substance :

Result : At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions.
Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: no data
Lot No.: no data
Supplier: Shell Chemical Co.
Reliability : (4) not assignable

Insufficient experimental detail
05.05.2004 (2)

Type : LD50
Value : 2363 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1984
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 1965 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1984
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 2700 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1982
GLP : no data
Test substance : no data

Source : Synthetic Chemical Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 1500 mg/kg bw

Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1982
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 2343 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1987
GLP : no data
Test substance :

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 1445 mg/kg bw
Species : guinea pig
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1987
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 12000 mg/m³
Species : rat
Strain : Sprague-Dawley

Sex	:	male/female
Number of animals	:	
Vehicle	:	other: aerosolised test substance
Doses	:	up to 12,000 mg/m3
Exposure time	:	4 hour(s)
Method	:	other: Andersen et al (1977)
Year	:	1977
GLP	:	no
Test substance	:	

Method : A modified acute inhalation chamber consisting of a 30 litre cylindrical glass battery jar supported in a plexiglas frame was used. Aerosols were generated using a glass nebuliser and final chamber concentrations were varied by altering the drop rate of sulfolane into the nebuliser. Particle size distribution analysis of the aerosol, collected with a six-stage cascade impactor, showed that 98% of the aerosol was between 1 and 4 µm in mean particle size diameter.

Exposures were made at various concentrations, all of which were below the aerosol concentration that which caused wetting of the animals and the chamber surfaces (approximately 12,000 mg/m3).

Result : At 12000 mg/m3 of sulfolane (the highest concentration that could be maintained as a stable aerosol) no rats died during 4 hour exposures or during a subsequent 2-week observation period.

Exposures at these high concentrations were continued until all all rats died. It was calculated that a mean survival time of 24 hours would be observed in atmospheres containing 4700 mg of the test substance per m3.

Nine rats were exposed to 3600 mg/m3. After 17.5 hours of exposure, when all nine had convulsed and were in extremis, the rats were euthanised and blood collected for haematological analysis. Significant decreases in white blood cell counts were observed, but haemocrit (HCT) and haemoglobin (HGB) were unchanged. At necropsy all rats exhibited pulmonary haemorrhage, although the severity of haemorrhage varied greatly.

Test condition : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
05.05.2004 (3)

Type	:	other
Value	:	ca. 4850 mg/m³
Species	:	monkey
Strain	:	other: squirrel monkey
Sex	:	male
Number of animals	:	2
Vehicle	:	other: aerosolised test substance
Doses	:	4850 mg/m3
Exposure time	:	18.5 hour(s)
Method	:	other: Andersen et al (1977)
Year	:	1977
GLP	:	no
Test substance	:	

Result	:	Two squirrel monkeys exposed to 4850 mg/m3 vomited and convulsed during exposure and were sacrificed after 18.5 hours. Both had greater than 25% reduction in white blood cell counts and greater than 15% reduction in HGB and HCT. Their lungs were haemorrhagic.
Test condition	:	Name: Sulfolane (CAS No. 126-33-0) Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water Lot No.: No data Supplier: Shell Chemical Co.
Reliability	:	(2) valid with restrictions

07.05.2004 (3)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw
Species	:	rat
Strain	:	other: Crl:CD.BR
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: test substance administered undiluted
Doses	:	2000 mg/kg
Method	:	other: 84/449/EEC, Method B3
Year	:	1993
GLP	:	yes
Test substance	:	
Result	:	Mortality: none Clinical signs: The majority of the rats showed yellow staining of the anogenital fur and one rat showed an unkempt appearance on Day 2 only. Erythema or yellow discolouration of the sites of application of Sulfolane were common after removal of the dressings on Day 2. All dermal changes resolved by Day 4. All rats had gained weight relative to their Day 1 bodyweights by the end of the first and second weeks of the 14 day observation period. Necropsy: No macroscopic changes were apparent at necropsy on Day 15.
Test condition	:	Age at study initiation: 5 weeks old for both sexes No. of animals/sex/group: 5 males. 5 females Mode of administration: Applied directly to clipped, intact skin using occlusive dressings.
Test substance	:	Name: Sulfolane (CAS No. 126-33-0) Purity: 97.4% Impurities: water (2.6%) Lot No.: AIS00057; Tank T1413 Supplier: Shell Chemical Co.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

07.05.2004 (12)

Type	:	LD50
Value	:	> 3800 mg/kg bw
Species	:	rat

Strain	:	Wistar
Sex	:	male/female
Number of animals	:	8
Vehicle	:	other:none
Doses	:	3800 mg/kg
Method	:	other: not specified
Year	:	1966
GLP	:	no
Test substance	:	
Result	:	No effects were observed in any of the rats.
Test condition	:	Undiluted sulfolane placed on the shorn dorsolumbar skin and bandaged into contact using an impermeable dressing of aluminium foil and water proof plaster.
Test substance	:	Name: Sulfolane (CAS No. 126-33-0) Purity: anhydrous Supplier: Shell Chemical Co.
Reliability	:	(4) not assignable Insufficient experimental detail

07.05.2004 (5)

Type	:	LD50
Value	:	12600 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	other: undiluted
Doses	:	6.8, 10.2, 15.4 and 23.1 g/kg/bw
Method	:	other: not specified
Year	:	1990
GLP	:	no data
Test substance	:	no data
Result	:	Clinical observations: weakness, lethargy, paralysis of the hind quarters and moderate to severe tremors. All deaths occurred within two days after the start of the application. Severe erythema and oedema were noted at the application site. Necropsy: No gross pathological alterations were noted.
Test condition	:	Animals were dosed with the undiluted test substance under an occlusive plastic sheet. Contact was maintained for 24 hours on shaved skin and observations made over 14 days.
Test substance	:	Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (9)

Type	:	LD50
Value	:	4897 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	1984
GLP	:	no data

Test substance :

Remark : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 4897 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1969
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

Type : LD50
Value : 12600 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year : 1984
GLP : no data
Test substance :

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : other: acute behavioural toxicity
Value :
Species : rat
Strain : Long-Evans
Sex : no data
Number of animals : 80
Vehicle : physiol. saline
Doses : 0, 200, 400, 800 mg/kg
Route of admin. : i.p.
Exposure time : 1 hour(s)
Method : other: Ruppert & Dyer, 1985
Year : 1985
GLP : no data

Test substance	:	
Method	:	Adult male rats (Long-Evans), 10 per group, received a single interperitoneal injection of saline, 200, 400 or 800 mg/kg sulfolane. Separate groups of rats at each dose were housed in rooms maintained at 32.3±0.7°C (warm ambient temperature) or 20.8±0.2°C (cool ambient temperature). Motor activity was assessed in figure of eight mazes one hour after dosing. Immediately after testing (one hour), body temperatures were recorded.
Result	:	At the cool ambient temperature, the body temperature of rats receiving 400 and 800 mg/kg was lower than that of the controls. At the warm ambient temperature, hypothermia in the rats receiving 400 and 800 mg/kg was attenuated, if not prevented. One animal receiving 800 mg/kg at the warm ambient temperature died during testing. At both ambient temperatures, 400 and 800 mg/kg sulfolane produced a decrease in motor activity. At the cool ambient temperature, 800 mg/kg sulfolane produced a decrease in movement throughout the maze. It was concluded that a behavioural change could be detected at sublethal dosages of sulfolane in the absence of hypothermia.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0) Purity: 99% Source: Aldrich Chemicals
Reliability	:	(2) valid with restrictions Reported in peer-reviewed literature

22.06.2004 (37)

Type	:	other: acute behavioural toxicity
Value	:	
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male
Number of animals	:	9
Vehicle	:	physiol. saline
Doses	:	0, 100, 200, 400, 600 and 750 mg/kg
Route of admin.	:	s.c.
Exposure time	:	
Method	:	other: Mohler & Gordon, 1988
Year	:	1988
GLP	:	no data
Test substance	:	
Method	:	Dose response:

A total of 9 male New Zealand White rabbits were used in this experiment. The rabbits were housed at an ambient temperature of 22°C.

During an experiment, individual rabbits were restrained and placed in an aluminium chamber that was immersed in a water bath maintained at a constant temperature of 10°C. Metabolic rate was calculated according to standard techniques using the rate of oxygen consumption. Colonic temperature of the rabbits was measured using a probe and ear skin temperature was measured using a thermocouple attached to the hairless side of the pinna. The ambient temperature was measured using a thermocouple secured to the wall of the chamber.

Solidified sulfolane was warmed to 37°C and then diluted with appropriate volumes of 0.9% saline to yiled dosages of 0, 100, 200 and 400 mg/kg in an injection volume of 1mL. Due to the limit of solubility of sulfolane, dosages of 600 and 750 mg/kg were obtained by increasing the injection volume.

Each rabbit received up to 3 separate exposures to sulfolane. Repeated

exposures to sulfolane were spaced at least one week apart, and no rabbit received the same dose twice.

Statistical analysis: Regression models were fit to the data (SAS 1986, SAS Institute Inc.) The data were divided into two subsets: (1) the first exposure for each animal (N=9) and (2) all exposures (N=17).

Ambient Temperature Effects:

A total of seven New Zealand White rabbits were used in this experiment. Housing conditions were as described above.

Rabbits were injected with 600 mg/kg sulfolane at ambient temperatures of 10, 20 or 28°C.

Each rabbit received up to 3 separate exposures to sulfolane. Repeated exposures to sulfolane were spaced at least one week apart, and no rabbit was tested at the same ambient temperature twice.

Statistical analysis: Data were divided into first exposure (N=7) and all exposures (N=12) subsets. Significance between the changes in colonic temperature at 10, 20 and 28°C was tested using conservative Bonferonni paired comparison t-tests. Changes in colonic and ear temperatures and metabolic rate at each ambient temperature were tested for significane using Student's t-test.

Result : Dose response:
Colonic and ear skin temperatures of the rabbit were significantly affected by subcutaneous administration of sulfolane and these effects were dose-dependent. The decrease in colonic temperature was linear, with a 0.13°C decrease per 100 mg/kg increase in sulfolane dose level. . Metabolic rate remained unchanged during the initial phase of the hypothermia for all dose groups; but peripheral vasodilation, as indicated by an increase in ear skin temperature, was seen at the higher dose levels.

It was noted that the highest doses of sulfolane caused behavioural deficits in the rabbits. Two to three hours after exposure to 600 mg/kg sulfolane, when the rabbits were removed from the environmental chamber and first observed, the animals exhibited a slight postural tremor similar to shivering. Both rabbits receiving 750 mg/kg sulfolane exhibited tonic seizures characterised by gross muscle contraction, forceful urination, and some vocalisation. These episodes were followed by exhaustion, panting, loss of postural control, and near catatonia. All rabbits in these experiments survived the sulfolane exposure, even at the highest dose levels.

Ambient Temperature Effects:

Subcutaneous injection of 600 mg/kg sulfolane caused a significant decrease in colonic temperature at ambient temperatures of 10 and 20°C, but to a significant increase in colonic temperature at 28°C. At ambient temperatures of 10 and 20°C, metabolic rate did not change significantly prior to or during peak hypothermia. Metabolic rate was significantly increased at 28°C.

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Reported in peer-reviewed literature

22.06.2004 (29)

Type : LD50

Value : 1598 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.p.
Exposure time :
Method : other: Andersen et (1976)
Year : 1976
GLP : no
Test substance : no data
Result : Rats assumed a hunched, retreating posture with front limbs braced wide, tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions. In the post-ictal period rats were unresponsive to loud noises and pain, resisted changes in position with only a weak righting reflex and had a weak corneal response. These toxic signs were attributed to a CNS excitatory effect, followed by a period of post-ictal depression.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 1250 mg/kg bw
Species : mouse
Strain : other: albino mongrel
Sex : male/female
Number of animals : 8
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other: Kamalova et al (1979)
Year : 1979
GLP : no
Test substance :
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (17)

Type : LD50
Value : 1270
Species : mouse
Strain : Swiss
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.p.
Exposure time :
Method : other: Andersen et al. (1976)
Year : 1976

GLP : no
Test substance :
Result : Mice assumed a hunched, retreating posture with front limbs responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 1331 mg/kg bw
Species : guinea pig
Strain : Hartley
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.p.
Exposure time :
Method : Andersen et al. (1976)
Year : 1976
GLP : no
Test substance :
Result : At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 3.18 ml/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : s.c.
Exposure time :
Method : other: Smyth et al. (1969)
Year : 1969
GLP : no
Test substance :
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (39)

Type : LD50
Value : 1606 mg/kg bw
Species : rat
Strain : Sprague-Dawley

Sex	:	male
Number of animals	:	
Vehicle	:	no data
Doses	:	no data
Route of admin.	:	s.c.
Exposure time	:	
Method	:	other: Andersen et al. (1976)
Year	:	1976
GLP	:	no
Test substance	:	
Result	:	Rats assumed a hunched, retreating posture with front limbs braced wide, tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions. In the post-ictal period rats were unresponsive to loud noises and pain, resisted changes in position with only a weak righting reflex and had a weak corneal response. These toxic signs were attributed to a CNS excitatory effect, followed by a period of post-ictal depression.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0) Supplier: Shell Chemical Co.
Reliability	:	(4) not assignable Insufficient experimental detail

22.06.2004 (2)

Type	:	LD50
Value	:	1360
Species	:	mouse
Strain	:	Swiss
Sex	:	male
Number of animals	:	
Vehicle	:	no data
Doses	:	no data
Route of admin.	:	s.c.
Exposure time	:	
Method	:	other: Andersen et al. (1976)
Year	:	1976
GLP	:	no
Test substance	:	
Result	:	Mice assumed a hunched, retreating posture with front limbs braced wide, tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0) Supplier: Shell Chemical Co.
Reliability	:	(4) not assignable Insufficient experimental detail

22.06.2004 (2)

Type	:	LD50
Value	:	1900 - 3500 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male
Number of animals	:	
Vehicle	:	no data
Doses	:	no data

Route of admin. : s.c.
Exposure time :
Method : Andersen et al. (1976)
Year : 1976
GLP : no
Test substance :
Result : At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudates becoming frothy and blood-tinged following convulsions.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 1094
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.v.
Exposure time :
Method : other: Andersen et al. (1976)
Year : 1976
GLP : no
Test substance :
Result : Rats assumed a hunched, retreating posture with front limbs braced wide, tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions. In the post-ictal period rats were unresponsive to loud noises and pain, resisted changes in position with only a weak righting reflex and had a weak corneal response. These toxic signs were attributed to a CNS excitatory effect, followed by a period of post-ictal depression.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.
Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 632 mg/kg bw
Species : mouse
Strain : Swiss
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.v.
Exposure time :
Method : Andersen et al. (1976)
Year : 1976
GLP : no
Test substance :
Result : Mice assumed a hunched, retreating posture with front limbs braced wide,

tail erect. Hyperreactivity, increased responsiveness to auditory stimulation, and rapid respiration were noted. At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudate becoming frothy and blood-tinged following convulsions.

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 1080 mg/kg bw
Species : mouse
Strain : Swiss Webster
Sex : male/female
Number of animals :
Vehicle : water
Doses : no data
Route of admin. : i.v.
Exposure time :
Method : other: Alexander et al. (1959)
Year : 1959
GLP : no
Test substance :
Result : The mice first showed restlessness, then hyper-responsiveness to auditory and tactile stimuli, followed by a sudden start and, in rapid succession, a typical squeal and clonic convulsions which were followed by periods of post-ictal depression before the recurrence of convulsions.

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)

Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (1)

Type : LD50
Value : 640 - 850 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals :
Vehicle : no data
Doses : no data
Route of admin. : i.v.
Exposure time :
Method : other: Andersen et al. (1976)
Year : 1976
GLP : no
Test substance :
Result : At lethal doses clonic-tonic convulsions which were precipitated by loud noises. Clear fluids exuded from the eyes and nares prior to convulsions with the exudates becoming frothy and blood-tinged following convulsions.

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient experimental detail

22.06.2004 (2)

Type : LD50
Value : 1270 mg/kg bw
Species : mouse

Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1986
GLP	:	no data
Test substance	:	no data
Remark	:	Mortality varied directly with ambient temperature 75% mortality at 35°C 8% mortality at 25°C
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

22.06.2004 (16)

Type	:	other
Value	:	400 - 800 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1986
GLP	:	no data
Test substance	:	
Remark	:	Visual disturbances and hypothermia observed.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

22.06.2004 (16)

Type	:	other
Value	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1988
GLP	:	
Test substance	:	
Remark	:	An increase in seizure sensitivity detected.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

22.06.2004 (16)

Type	:	LD50
Value	:	1080 mg/kg bw

Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.v.
Exposure time	:	
Method	:	
Year	:	1982
GLP	:	no data
Test substance	:	no data
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

22.06.2004 (16)

5.2.1 SKIN IRRITATION

Species	:	guinea pig
Concentration	:	undiluted
Exposure	:	Open
Exposure time	:	
Number of animals	:	10
Vehicle	:	other: none
PDII	:	
Result	:	not irritating
Classification	:	not irritating
Method	:	other: Brown et al. (1966)
Year	:	1966
GLP	:	no
Test substance	:	
Result	:	No gross appearance of skin irritation or microscopic changes.
Test condition	:	Dosing occurred daily, five times a week for four and a half weeks (23 applications). 0.5 ml of substance used per dose.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Reported in peer-reviewed literature

22.06.2004 (5)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	
Number of animals	:	8
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	not irritating
Method	:	other: Brown et al. (1966)
Year	:	1966
GLP	:	no
Test substance	:	
Result	:	No signs of skin irritation were observed in any of the rabbits used, and the total erythema score was less than 10 and can be ignored. Histopathological examination of skins taken from the rabbits revealed no

evidence of skin damage.
Test condition : Rabbit exposure for 6 hours per day over 3 days.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (2) valid with restrictions
Reported in peer-reviewed literature
22.06.2004 (5)

Species : rabbit
Concentration : undiluted
Exposure : Open
Exposure time :
Number of animals : 2
Vehicle : other: none
PDII :
Result : not irritating
Classification : not irritating
Method : other: Brown et al. (1966)
Year : 1966
GLP : no
Test substance :

Result : No gross appearance of skin irritation or microscopic changes.
Test condition : Dosing occurred daily, five times a week for four and a half weeks (23 applications). 1 ml of substance used per dose.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (3) invalid
22.06.2004 (5)

Species : rabbit
Concentration : undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 4
Vehicle : other: none
PDII : .7
Result : not irritating
Classification : not irritating
Method : other: not specified
Year : 1990
GLP : no data
Test substance :

Test condition : Undiluted test material was applied to the shaved backs of four albino rabbits at a volume of 0.5 ml on each test site. The material was confined to shaved, abraded and intact skin sites having an area of one inch square under a gauze patch. The entire trunk was enclosed with an occlusive plastic sheet and contact was maintained for 24 hours.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
22.06.2004 (9)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : .2 ml
Exposure time : unspecified
Comment :

Number of animals	:	
Vehicle	:	none
Result	:	not irritating
Classification	:	not irritating
Method	:	other: US Federal Register (1964)
Year	:	1966
GLP	:	no
Test substance	:	no data

Result	:	Mild conjunctivitis cleared within a few hours.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions

07.05.2004 (5)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	other: Draize method
Number of animals	:	5
Vehicle	:	none
Result	:	slightly irritating
Classification	:	not irritating
Method	:	Draize Test
Year	:	1990
GLP	:	no data
Test substance	:	

Result	:	Corneal opacity was noted in all rabbits but by 72 hours post-instillation it had cleared in all animals but one. The conrnea of this latter animal was cleared by Day 7. Iridial irritancy and slight conjunctival irritancy were noted through 72 to 96 hours. By Day 7 post-installation all eyes appeared normal.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(4) not assignable

07.05.2004 (9)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	moderately irritating
Classification	:	irritating
Method	:	
Year	:	1969
GLP	:	no data
Test substance	:	no data

Remark	:	Corneal injury, Graded 4 on a scale of 1-10.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable

17.06.2004 (16)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 2 % intracutaneous 2nd: Challenge undiluted occlusive epicutaneous 3rd:
Number of animals	:	34
Vehicle	:	water
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	Directive 84/449/EEC, B.6 "Acute toxicity (skin sensitization)"
Year	:	1993
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	None of the twenty test animals showed any positive response at either 24 or 48 hours after removal of the challenge patches. It may be concluded therefore, that the substance is not a skin sensitiser in guinea pigs. Necropsy did not elucidate the cause of death of a male control animal found dead thirteen days after intradermal induction; macroscopic changes were limited to slight dilatation of the samll intestine, large intestine and caecum.
Test condition	:	Intrademal induction: 2% m/v Sulfolane in water/FCA Topical induction: undiluted Sulfolane Topical challenge: undiluted Sulfolane A group of 10 male and 10 female guinea pigs were induced intradermally using 2% m/v sulfolane in water/Freunds Complete Adjuvant followed a week later by topical induction using undiluted sulfolane (0.3 ml) which was applied over the sites of the intradermal injections and covered occlusively for 48 hours. Challenge was carried out 3 weeks after the intradermal induction. Undiluted sulfolane (0.1ml) was applied to the shaven backs of the test animals and covered with occlusive tape for 24 hours. Dermal reaction to the challenge was assessed after removal of the bandages and at 24 hours and 48 hours after challenge.
Reliability	:	(1) valid without restriction

07.05.2004 (12)

Type	:	no data
Species	:	guinea pig
Concentration	:	1st: Induction .1 % 2nd: Challenge .1 % 3rd:
Number of animals	:	
Vehicle	:	no data
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	other: Brown et al (1966)
Year	:	1966
GLP	:	no
Test substance	:	
Method	:	The test was carried out by injecting or applying a 0.1% w/v of the chemical to the shorn skin on three days, in each of three successive weeks. No treatment for the next 10 days, and then a challenge dose of the same solution was applied. Animals examined at 1, 24 and 48 hours after challenge for signs of a sensitization-type reaction.

The procedure was repeated using the same animals.

Result : Sulfolane did not produce signs of sensitisation in either topical or intradermal tests. The repeated tests also did not promote a sensitisation response.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (5)

Type : no data
Species : guinea pig
Number of animals : 40
Vehicle : other: acetone
Result : not sensitizing
Classification : not sensitizing
Method : other: not specified
Year : 1982
GLP : no data
Test substance :

Result : Guinea pigs receiving the test substance, 75% sulfolane in acetone, during the challenge phase only and those animals exposed to the same challenge dose following the induction with 75% sulfolane in acetone and a rest period exhibited no dermal irritation at 24, 48 or 72 hours.

Guinea pigs treated with the positive control (0.10% DNCB in acetone) during the challenge phase only exhibited very slight erythema in three animals at 24 hours which persisted in one animal to 48 hours. Animals exposed to the same challenge concentration of DNCB following induction with 0.15% DNCB in acetone and a rest period exhibited very slight to moderate to severe erythema at 24 hours and persisted to some degree to 48 and 72 hours in several animals; very slight oedema in one animal at 24 hours; and blanching in four animals at 24 hours. Comparison of these dermal responses indicate that the guinea pigs responded to hypersensitisation when a known sensitiser was used.
Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (30)

5.4 REPEATED DOSE TOXICITY

Type :
Species : rat
Sex : male/female
Strain : Crj: CD(SD)
Route of admin. : gavage
Exposure period : 28 Days
Frequency of treatm. : Daily
Post exposure period : 14 days (for 0 and 700 mg/kg/day group)
Doses : 0, 60, 200, 700 mg/kg/day
Control group : yes, concurrent vehicle
Method : other: Japanese TG
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : NOEL: Males 60 mg/kg/day, Females 200 mg/kg/day

LOAEL: Males 200 mg/kg/day, Females 700 mg/kg/day

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
Clinical signs: There was transient reduction of locomotor activity in the early stages in 700 mg/kg/day females.
Bodyweight and food consumtion: Decreased in 700 mg/kg/day males and females.
Mortality and time to death: There were no deaths.
Urinary examination: No changes considered to be attributable to the test substance.

Haematology and biochemical findings:
Males: Slight decrease in MCHC in all groups, but not dose-related. No changes in erythrocytes, haemoglobin and haemocrit values. Increase in leucocytes in the 700 mg/kg recovery group.
Females: Decrease in erythrocytes and increase in MCV in the 700 mg/kg recovery group.

See table 1.

Blood chemistry:
Males: Increase of cholinesterase and total bilirubin and decrease of chloride in the 700mg/kg recovery group.
Females: Increase of GPT and decrease of glucose in the 700mg/kg recovery group.

See table 2.

Gross pathology incidence and severity: Slight hypertrophy of kidney in 700 mg/kg recovery groups males (2/6)

Organ weight changes: Increase in relative weight of kidneys, brain and heart in 700 mg/kg recovery group males. Decrease in splenic weight, and increase of absolute and relative weight of spleen in 700 mg/kg recovery group females.

See table 3.

Histopathology: Males: Increase of hydraline droplets and eosinophilic bodies in renal tubules at 200 and 700 mg/kg. Increase of basophilic renal tubules at 700 mg/kg. Females: No significant effects were observed.

Test condition : TEST ORGANISMS:
Age at study initiation: 5 weeks old
No. of animals per sex per dose: 6 per sex per dose for 60 and 200 mg/kg/day groups. 12 per sex per dose for the 0 and 700 mg/kg/day groups.

Table 1: Significant haematological findings in male and female rats after oral administration of tetrahydrothiophene 1,1-dioxide for 28 days and a recovery period of 14 days

28 day dose group					14 day recovery group	
Dose level (mg/kg)	0	60	200	700	0	700
No. of animals	6	6	6	6	6	6
Male						
MCHC (%)	34.6 ± 0.8	33.8 ± 0.4*	33.5 ± 0.2**	33.6 ± 0.4**	24.3 ± 0.5	34.5 ± 0.8
leucocytes ($10^2/mm^3$)	60 ± 16	58 ± 19	58 ± 13	64 ± 7	76 ± 19	104 ± 22**

Female						
erythrocytes (10^4/mm^3)	773 ± 21	778 ± 32	752 ± 23	778 ± 42	817 ± 16	781 ± 21**
MCV (f/)	57 ± 2	57 ± 2	57 ± 1	58 ± 1	55 ± 1	57 ± 1**

(*P < 0.05, **P < 0.01)

Table 2: Significant blood chemistry findings in male and female rats after oral administration of tetrahydrothiophene 1,1-dioxide for 28 days and a recovery period of 14 days

28 day dose group					14 day recovery group	
Dose level (mg/kg)	0	60	200	700	0	700
No. of animals	6	6	6	6	6	6
Male						
ChE(IU/l)	304 ± 175	296 ± 106	281 ± 60	294 ± 41*	292 ± 81	263 ± 47
T.bilirubin (mg/d/)	0.35 ± 0.05	0.35 ± 0.05	0.40 ± 0.05	0.45 ± 0.03**	0.28 ± 0.02	0.30 ± 0.05
Cl(mEq/l)	104 ± 0	104 ± 1	104 ± 1	102 ± 1**	103 ± 2	103 ± 1
Female						
GPT(IU/l)	24 ± 5	24 ± 4	23 ± 3	35 ± 6**	27 ± 6	29 ± 5
Glucose (mg/d/)	130 ± 15	117 ± 13	123 ± 10	110 ± 4 *	139 ± 13	125 ± 10

(*P < 0.05, **P < 0.01)

Table 3: Significant absolute and relative organ weight findings in male and female rats after oral administration of tetrahydrothiophene 1,1-dioxide for 28 days and a recovery period of 14 days

28 day dose group					14 day recovery group	
Dose level (mg/kg)	0	60	200	700	0	700
No. of animals	6	6	6	6	6	6
Male, relative weight						
Brain (%)	0.62 ± 0.03	0.64 ± 0.03	0.64 ± 0.03	0.68 ± 0.05*	0.52 ± 0.04	0.54 ± 0.04
Kidneys (g%)	0.77 ± 0.04	0.80 ± 0.05	0.79 ± 0.05	0.94 ± 0.06**	0.67 ± 0.05	0.71 ± 0.08
Heart (g%)	0.34 ± 0.03	0.35 ± 0.03	0.35 ± 0.01	0.39 ± 0.03*	0.32 ± 0.02	0.34 ± 0.03
Female, absolute weight						
Spleen (g)	0.48 ± 0.06	0.43 ± 0.05	0.44 ± 0.08	0.37 ± 0.03*	0.44 ± 0.06	0.53 ± 0.05*
relative weight						
Spleen (g)	0.24 ± 0.03	0.22 ± 0.03	0.23 ± 0.05	0.20 ± 0.01	0.20 ± 0.02	0.24 ± 0.02*

(*P < 0.05, **P < 0.01)

Conclusion : The NOAELs are considered to be 60 mg/kg/day for males and 200 mg/kg/day for females.
The LOAELs are considered to be 200 mg/kg/day for males and 700 mg/kg/day for females.

Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP

Flag : Critical study for SIDS endpoint

07.05.2004 (24)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	27 days
Frequency of treatm.	:	8 hr/day, 5 days/week
Post exposure period	:	none
Doses	:	495 ± 75 mg/m3
Control group	:	no data specified
Method	:	other: Andersen et al. (1977)
Year	:	1977
GLP	:	no
Test substance	:	
Result	:	Toxic effects: Chronic lung inflammation and chronic liver inflammation. A slight but non-significant decrease in WBC
Test condition	:	TEST DESIGN: No. of animals: 8 males and 7 females Vehicle: Distilled water Clinical observations performed and frequency: Bodyweight, haematological and blood chemical analysis, and urinalysis at the end of the exposure period. Examinations at necropsy: Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta. Statistical analysis: Student's t-test and unknown
Test substance	:	Name: Sulfolane (CAS No. 126-33-0) Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water Lot No.: No data Supplier: Shell Chemical Co.
Reliability	:	(4) not assignable Insufficient deatil in available literature

07.05.2004 (3)

Type	:	
Species	:	guinea pig
Sex	:	male/female
Strain	:	Hartley
Route of admin.	:	inhalation
Exposure period	:	27 days
Frequency of treatm.	:	8 hrs/day, 5 days/week
Post exposure period	:	none
Doses	:	495 ± 75 mg/m3
Control group	:	no data specified
Method	:	other: Andersen et al. (1977)
Year	:	1977
GLP	:	no
Test substance	:	
Result	:	Toxic effects: Chronic lung inflammation
Test condition	:	TEST DESIGN: No. of animals: 8 males and 7 females Vehicle: Distilled water Clinical observations performed and frequency: Bodyweight,

haematological and blood chemical analysis, and urinalysis at the end of the exposure period.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (3)

Type :
Species : dog
Sex :
Strain : Beagle
Route of admin. : inhalation
Exposure period : 27 days
Frequency of treatm. : 8 hrs/day, 5 days/week
Post exposure period : none
Doses : 495 ± 75 mg/m3
Control group : no data specified
Method : other: Anderson et al. (1977)
Year : 1977
GLP : no
Test substance :

Result : Chronic lung inflammation
Test condition : TEST DESIGN:
No. of animals: 2 males
Vehicle: Distilled water
Clinical observations performed and frequency: Bodyweight, haematological and blood chemical analysis at the end of the exposure period.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (3)

Type :
Species : monkey
Sex : male
Strain : other: squirrel monkey
Route of admin. : inhalation
Exposure period : 27 days

Frequency of treatm.	:	8 hrs/day, 5 days week
Post exposure period	:	none
Doses	:	495 ± 75 mg/m3
Control group	:	no data specified
Method	:	other: Andersen et al. (1977)
Year	:	1977
GLP	:	no
Test substance	:	
Result	:	Toxic effects: 3 animals died and 5 were sacrificed during exposure. Blood-tinged fluid around the eyes and very pale liver and heart. Fatty metamorphosis of the liver and chronic liver inflammation observed. A slight but non-significant decrease in WBC was observed.
Test condition	:	TEST DESIGN: No. of animals: 9 males Vehicle: Distilled water Clinical observations performed and frequency: Bodyweight, haematological and blood chemical analysis at the end of the exposure period. Examinations at necropsy: Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta. Statistical analysis: Student's t-test and unknown
Test substance	:	Name: Sulfolane (CAS No. 126-33-0) Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water Lot No.: No data Supplier: Shell Chemical Co.
Reliability	:	(4) not assignable Insufficient detail in available literature

07.05.2004 (3)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	90 -110 days
Frequency of treatm.	:	23 hrs/day
Post exposure period	:	none
Doses	:	2.8 ± 1.4 mg/m3 (90 days), 4.0 ± 1.0 mg/m3 (110 days), 20 ± 6.7 mg/m3 (95 days)
Control group	:	no data specified
NOAEL	:	20 mg/m³
Method	:	other: Andersen et al. (1977)
Year	:	1977
GLP	:	no
Test substance	:	
Remark	:	2/7 female rats in the 20 mg/m3 group had slightly elevated AsT, AIT and LD activities. The importance of such an observation is uncertain.
Result	:	NOAEL = 20 mg/m3
Test condition	:	TEST DESIGN: No. of animals: 8 males and 7 females for 20 mg/m3 15 males for 2.8 and 4.0 mg/m3

Vehicle: Distilled water
Clinical observations performed and frequency: Bodyweight, haematological and blood chemical analysis, and urinalysis at the end of the exposure period.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Non-standard method, insufficient detail in avaialble literature

07.05.2004 (3)

Type :
Species : rat
Sex :
Strain : no data
Route of admin. : other: no data
Exposure period : 4 months
Frequency of treatm. : no data
Post exposure period : no data
Doses : 5.1 g/kg
Control group : no data specified
Method : other: Filippova et al (1968)
Year : 1968
GLP : no
Test substance :

Result : After four months no bodyweight changes, no blood dyscrasis, no blood pressure changes and no liver function changes.

Test substance : Chemical Name: Sulfolane (CAS No. 126-33-0)

Reliability : (4) not assignable
Insufficient detail available in literature

07.05.2004 (11)

Type :
Species : dog
Sex : male
Strain : Beagle
Route of admin. : inhalation
Exposure period : 90 - 110 days
Frequency of treatm. : 23 hr/day
Post exposure period : none
Doses : 2.8 ± 1.4 mg/m3 for 90 days , 4.0 ± 1.0 mg/m3 for 110 days, 20 ± 6.7 mg/m3 for 95 days, 200 ± 48 mg/m3 for 90 days
Control group : no data specified
NOAEL : 20 mg/m³
LOAEL : 200 mg/m³
Method : other: Andersen et al. (1977)
Year : 1977
GLP : no
Test substance :

Result : Toxic effects: Convulsion, fierce aggressive behaviour,

motor seizure, vomitting, chronic pulmonary inflammation.

Test condition : TEST DESIGN:
No. of animals: 4, 2, 1 and 1 for the 200, 20, 4.0 and 2.8 mg/m3 groups respectively
Vehicle: Distilled water
Clinical observations performed and frequency: Bodyweight determined after 30 and 60 days and at the end of the exposure period, haematological and blood chemical analysis carried out at the end of the exposure period.
Heamatological analysis was also carried out at the 30 and 60 day time points.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (3)

Type :
Species : guinea pig
Sex : male/female
Strain : Hartley
Route of admin. : inhalation
Exposure period : 85 - 110 days
Frequency of treatm. : 23 hrs/day
Post exposure period : none
Doses : 2.8 ± 1.4 mg/m3 for 90 days, 4.0 ± 1.0 mg/m3 for 110 days, 20 ± 6.7 mg/m3 for 95 days, 159 ± 68 mg/m3 for 85 days, 200 ± 48 mg/m3 for 90 days
Control group : no data specified
Method : other: Andersen et al. (1977)
Year : 1977
GLP : no
Test substance :

Result : NOAEL: 159 mg/m3
LOAEL: 200 mg/m3
Toxic effects: Fatty vacuolization in the liver. Decrease in WBC counts.

Test condition : TEST DESIGN:
No. of animals: 15 of each sex for 200 mg/m3
24 of each sex for 159 mg/m3
8 males and 7 females for 20 mg/m3
15 males for 2.8 and 4.0 mg/m3 groups
Vehicle: Distilled water
Clinical observations performed and frequency: Bodyweight determined after 30 and 60 days and at the end of the exposure period, haematological and blood chemical analysis, and urinalysis at the end of the exposure period.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum,

oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (3)

Type :
Species : monkey
Sex : male
Strain : other: squirrel monkey
Route of admin. : inhalation
Exposure period : 90 - 110 days
Frequency of treatm. : 23 hrs/day
Post exposure period : none
Doses : 2.8 ± 1.4 mg/m3 for 90 days, 4.0 ± 1.0 mg/m3 for 110 days, 20 ± 6.7 mg/m3 for 95 days, 200 ± 48 mg/m3 for 90 days
Control group : no data specified
NOAEL : 20 mg/m³
LOAEL : 200 mg/m³
Method : other: Andersen et al. (1977)
Year : 1977
GLP : no
Test substance :

Result : Toxic effects: Two monkeys treated with 200 mg/m3 died or became moribund within the first 7 days. Chronic pleuritis noted.

Test condition : TEST DESIGN:
No. of animals: 2, 6, 9 and 9 for the 200, 20, 4.0 and 2.8 mg/m3 groups respectively
Vehicle: Distilled water
Clinical observations performed and frequency: Bodyweight determined after 30 and 60 days and at the end of the exposure period, haematological and blood chemical analysis carried out at the end of the exposure period.
Heamatological analysis was also carried out at the 30 and 60 day time points.
Examinations at necropsy:
Microscopic: lung, bronchus, heart, kidney, bile duct, liver, spleen, stomach, intestine, pancreas, cerebellum, oesophagus, thyroid, trachea, lymph nodes, bladder and aorta.
Statistical analysis: Student's t-test and unknown

Test substance : Name: Sulfolane (CAS No. 126-33-0)
Purity: Redistilled from commercial product (Sulfolane-W) containing 3% water
Lot No.: No data
Supplier: Shell Chemical Co.

Reliability : (4) not assignable
Insufficient detail in available literature

07.05.2004 (3)

Type :
Species : rabbit

Sex	:	male/female
Strain	:	New Zealand white
Route of admin.	:	dermal
Exposure period	:	4 1/2 weeks
Frequency of treatm.	:	daily, 5 days/week
Post exposure period	:	
Doses	:	1 ml
Control group	:	no data specified
Method	:	
Year	:	1966
GLP	:	no data
Test substance	:	
Remark	:	No signs of skin irritation observed following repeated applications.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test substance	:	Undiluted.
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

Type	:	
Species	:	guinea pig
Sex	:	male/female
Strain	:	
Route of admin.	:	dermal
Exposure period	:	4 1/2 weeks
Frequency of treatm.	:	daily, 5 days/week
Post exposure period	:	
Doses	:	0.5 ml
Control group	:	no data specified
Method	:	
Year	:	1966
GLP	:	no data
Test substance	:	no data
Remark	:	No signs of skin irritation observed following repeated applications.
Source	:	Synthetic Chemicals Ltd. Wolverhampton
Test substance	:	Undiluted
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium TA100, TA1535, TA98, TA1537 Escherichia coli WP2uvrA
Test concentration	:	See test condition
Cycotoxic concentr.	:	see result
Metabolic activation	:	with and without
Result	:	negative
Method	:	OECD Guide-line 471, 472
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	CYTOTOXIC CONCENTRATION: Toxicity was not observed up to 5000µg/plate in the five

strains with and without S9 mix.
Precipitation was not observed at any concentration with and without S9 mix.
The substance did not induce mutations in the strains tested in either the presence or absence of metabolic activation.

See tables 1 and 2.

Test condition : DOSES IN ABSENCE AND PRESENCE OF ACTIVATION:
0, 313, 625, 1250, 2500 and 5000 µg/plate

METABOLIC ACTIVATION: S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone

POSITIVE CONTROLS:
-S9 mix; 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2), sodium azide (TA1535) and 9-aminoacridine (TA1537)
+S9 mix; 2-aminoanthracene (all five strains)

PLATES/TEST: 3
REPLICATES: 2

Table 1: Mutagenicity of tetrahydrothiophene 1,1-dioxide in a reverse mutation test on bacteria. Test 1.

With (+) or without (-) S9 mix	Test substance dose (µg/plate)	Number of revertants (number of colonies/plate, Mean ± S.D.)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9 mix (-)	0	90 88 89 (89 ± 1)	13 10 17 (13 ± 3.5)	20 22 24 (22 ± 2.0)	23 25 21 (23 ± 2.0)	9 10 10 (10 ± 0.6)
	313	124 107 103 (111 ± 11.2)	10 8 14 (11 ± 3.1)	25 35 36 (32 ± 6.1)	22 14 27 (21 ± 6.6)	11 7 8 (9 ± 2.1)
	625	103 131 120 (118 ± 14.1)	15 10 13 (13 ± 2.5)	36 33 27 (32 ± 4.6)	21 16 15 (17 ± 3.2)	9 11 6 (9 ± 2.5)
	1250	109 118 97 (108 ± 10.5)	7 13 15 (12 ± 4.2)	27 23 31 (27 ± 4.0)	15 18 22 (18 ± 3.5)	8 7 12 (9 ± 2.6)
	2500	87 142 124 (118 ± 28.0)	9 10 13 (11 ± 2.1)	27 27 23 (26 ± 2.3)	26 19 15 (20 ± 5.6)	6 6 11 (8 ± 2.9)
	5000	109 123 106 (113 ± 9.1)	9 12 9 (10 ± 1.7)	23 28 28 (26 ± 2.9)	24 26 15 (22 ± 5.9)	9 10 9 (9 ± 0.6)
S9 mix (+)	0	134 135 129 (133 ± 3.2)	9 16 18 (14 ± 4.7)	36 28 24 (29 ± 6.1)	30 32 38 (33 ± 4.2)	15 12 10 (12 ± 2.5)
	313	95 85 117 (99 ± 16.4)	7 10 10 (9 ± 1.7)	42 35 35 (37 ± 4.0)	46 40 33 (40 ± 6.5)	8 10 16 (11 ± 4.2)
	625	134 134 110 (126 ± 13.9)	13 11 12 (12 ± 1.0)	39 38 31 (36 ± 4.4)	33 38 33 (35 ± 2.9)	18 20 18 (19 ± 1.2)
	1250	114 131 119 (121 ± 8.7)	14 14 13 (14 ± 0.6)	27 19 24 (23 ± 4.0)	34 31 25 (30 ± 4.6)	16 8 14 (13 ± 4.2)
	2500	122 127 159 (136 ± 20.1)	5 13 5 (8 ± 4.6)	28 28 30 (29 ± 1.2)	30 33 42 (35 ± 6.2)	14 12 14 (13 ± 1.2)
	5000	149 136 128 (138 ± 10.6)	10 16 17 (14 ± 3.8)	19 17 23 (20 ± 3.1)	30 36 30 (32 ± 3.5)	12 10 14 (12 ± 2.0)
Positive control S9 mix (-)	Chemical	AF2	SA	AF2	AF2	9AA

	Dose (µg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	858 879 838 (858 ± 20.5)	193 193 206 (197 ± 7.5)	122 209 194 (175 ± 46.5)	735 788 820 (781 ± 42.9)	1767 2148 2578 (2164 ± 405.7)
Positive control S9 mix (+)	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Number of colonies/plate	1370 1381 1290 (1347 ± 49.7)	298 281 294 (291 ± 8.9)	1243 1223 1506 (1324 ± 157.9)	337 335 335 (336 ± 1.2)	287 280 283 (283 ± 3.5)

AF2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA: Sodium azide, 9AA: 9-Aminoacridine, 2AA: 2-Aminoanthracene

Table 2: Mutagenicity of tetrahydrothiophene 1,1-dioxide in a reverse mutation test on bacteria. Test 2.

With (+) or without (-) S9 mix	Test substance dose (µg/plate)	Number of revertants (number of colonies/plate, Mean ± S.D.)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9 mix (-)	0	112 123 125 (120 ± 7.0)	11 18 16 (15 ± 3.6)	17 18 33 (23 ± 9.0)	22 24 32 (26 ± 5.3)	12 7 8 (9 ± 2.6)
	313	131 127 156 (138 ± 15.7)	15 17 17 (16 ± 1.2)	23 31 32 (29 ± 4.9)	21 14 23 (19 ± 4.7)	9 17 5 (10 ± 6.1)
	625	137 130 128 (132 ± 4.7)	18 16 25 (20 ± 4.7)	29 24 22 (25 ± 3.6)	21 19 22 (21 ± 1.5)	12 8 10 (10 ± 2.0)
	1250	105 132 129 (122 ± 14.8)	20 16 15 (17 ± 2.6)	23 22 18 (21 ± 2.6)	27 24 27 (26 ± 1.7)	11 15 6 (11 ± 4.5)
	2500	137 145 133 (138 ± 6.1)	9 18 12 (13 ± 4.6)	28 20 23 (24 ± 4.0)	38 21 20 (26 ± 10.1)	14 9 14 (12 ± 2.9)
	5000	156 129 123 (136 ± 17.6)	25 20 15 (20 ± 5.0)	28 17 28 (24 ± 6.4)	27 23 31 (27 ± 4.0)	17 12 5 (11 ± 6.0)
S9 mix (+)	0	148 120 141 (136 ± 14.6)	15 12 21 (16 ± 4.6)	16 25 25 (22 ± 5.2)	35 39 20 (31 ± 10.0)	15 14 17 (15 ± 1.5)
	313	127 137 146 (137 ± 9.5)	22 26 14 (21 ± 6.1)	30 30 25 (28 ± 2.9)	42 31 39 (37 ± 5.7)	12 20 20 (17 ± 4.6)
	625	137 146 133 (137 ± 9.5)	15 10 16 (14 ± 3.2)	24 29 25 (26 ± 2.6)	29 38 42 (36 ± 6.7)	14 18 18 (17 ± 3.8)
	1250	137 142 143 (141 ± 3.2)	17 16 19 (17 ± 1.5)	33 25 26 (28 ± 4.4)	26 35 27 (29 ± 4.9)	20 19 13 (17 ± 3.8)
	2500	138 172 166 (159 ± 18.1)	17 15 18 (17 ± 1.5)	29 22 22 (24 ± 4.0)	38 39 29 (35 ± 5.5)	23 25 15 (21 ± 5.3)
	5000	134 132 159 (142 ± 15.0)	23 17 19 (20 ± 3.1)	19 22 28 (23 ± 4.6)	47 30 36 (38 ± 8.6)	9 16 13 (13 ± 3.5)
Positive control S9 mix (-)	Chemical	AF2	SA	AF2	AF2	9AA
	Dose (µg/plate)	0.01	0.5	0.01	0.1	80

	Number of colonies/ plate	728 781 743 (751 ± 27.3)	207 153 128 (163 ± 40.4)	85 81 91 (86 ± 5.0)	767 780 762 (770 ± 9.3)	908 890 965 (921 ± 39.2)
Positive control S9 mix (+)	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Number of colonies/ plate	1228 1435 1409 (1357 ± 112.8)	184 198 197 (193 ± 7.8)	1211 1206 1296 (1238 ± 50.6)	340 323 309 (324 ± 15.5)	204 192 221 (206 ± 14.6)

AF2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA: Sodium azide, 9AA: 9-Aminoacridine, 2AA: 2-Aminoanthracene

Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP
Flag : Critical study for SIDS endpoint
07.05.2004 (25)

Type : Chromosomal aberration test
System of testing : Chinese Hamster CHL/IU cell
Test concentration : 0.30, 0.60 and 1.2 mg/ml
Cycotoxic concentr. : 50% growth inhibition not observed at any concentration with or without S9 mix
Metabolic activation : with and without
Result : negative
Method : Guidelines for screening mutagenicity testing of chemicals, JAPAN
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : Structural chromosomal aberrations and polyploidy were not induced up to a maximum concentration of 1.2 mg/ml in continuous or short term treatment with and without an exogeneous metabolic activation system, respectively.

See tables 1 and 2.

Test condition : TEST DESIGN:
For continuous treatment, cells were treated for 24 or 48 hours without S9 mix. For short-term treatment, cells were treated for 6 hours with and without S9 mix and cultivated with fresh media for 18 hours.
Plates/test: 2
Positive controls: Mitomycin C for continuous treatment
Cyclophosphamide for short-term treatment
Solvent: water

Table 1: Chromosome analysis of Chinese hamster cell (CHL/IU) continuously treated with tetrahydrothiophene 1,1-dioxide (THTD)* without S9 mix

Group	Concen-tration (mg/ml)	Time of exposure (h)	No of cells analysed	No. of structural aberrations							others[3]	No. of cells with aberrations				Polyploid[4] (%)	Trend Test[5]	
				gap	ctb	cte	csb	cse	mul[2]	total		TAG	(%)	TA	(%)		SA	NA
Control			200	1	0	1	0	0	0	2	0	2	(1.0)	1	(0.5)	0.13	NT	NT
Solvent[1]	0	24	200	0	0	0	0	1	0	1	1	1	(0.5)	1	(0.5)	0.25		
THTD	0.30	24	200	1	0	0	0	0	0	1	0	1	(0.5)	0	(0.0)	0.13		
THTD	0.60	24	200	0	1	0	0	1	0	2	0	2	(1.0)	2	(1.0)	0.13		
THTD	1.2	24	200	1	0	0	0	0	0	1	0	1	(0.5)	0	(0.0)	0.63		
MC	0.00005	24	200	10	67	140	0	1	0	218	0	119	(59.5)	116	(58.0)	0.00		
Solvent[1]	0	48	200	1	0	1	2	0	0	4	0	3	(1.5)	2	(1.0)	0.50	NT	NT
THTD	0.30	48	200	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.00		
THTD	0.60	48	200	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.00		
THTD	1.2	48	200	1	0	0	0	0	0	1	0	1	(0.5)	0	(0.0)	0.25		
MC	0.00005	48	200	9	86	200	1	10	90	396	9	138	(69.0)	138	(69.0)	0.38		

Table 2: Chromosome analysis of Chinese hamster cell (CHL/IU) treated with tetrahydrothiophene 1,1-dioxide (THTD)* with and without S9 mix

Group	Concen-tration (mg/ml)	S9 mix	Time of exposure (h)	No of cells analysed	No. of structural aberrations							others[3]	No. of cells with aberrations				Polyploid[4] (%)	Trend Test[5]	
					gap	ctb	cte	csb	cse	mul[2]	total		TAG	(%)	TA	(%)		SA	NA

Control				200	2	0	1	0	0	0	3	0	3	(1.5)	1	(0.5)	0.63	NT NT
Solvent[1)]	0	-	6-(18)	200	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.00	
THTD	0.30	-	6-(18)	200	1	0	0	0	0	0	1	0	1	(0.5)	0	(0.0)	0.13	
THTD	0.60	-	6-(18)	200	1	1	0	0	0	0	2	0	2	(1.0)	1	(0.5)	0.25	
THTD	1.2	-	6-(18)	200	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.63	
CPA	0.005	-	6-(18)	200	1	0	1	0	0	0	2	0	2	(1.0)	1	(0.5)	0.50	
Solvent[1)]	0	+	6-(18)	200	1	1	1	0	0	0	3	0	3	(1.5)	2	(1.0)	0.63	NT NT
THTD	0.30	+	6-(18)	200	1	1	0	0	0	0	2	0	2	(1.0)	1	(0.5)	0.38	
THTD	0.60	+	6-(18)	200	0	0	0	0	1	0	1	0	1	(0.5)	1	(0.5)	0.13	
THTD	1.2	+	6-(18)	200	2	3	3	0	0	0	8	0	3	(1.5)	1	(0.5)	0.00	
CPA	0.005	+	6-(18)	200	2	161	311	0	4	440	918	0	186	(93.0)	186	(93.0)	0.00	

Abbreviations:
gap; chromatid gap and chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange (dicentric and ring etc.), mul: multiple aberrations, TAG: total no. of cells with aberrations, TA: total no. of cells with aberrations except gap, SA: structural aberration, NA: numerical aberration, MC: mitomycin C, NT: not tested.

Water for injection was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Cochran . Armitage's trend test was done at $p < 0.05$ when the incidence of TAG and polyploid in the treatment groups was significantly different from historical solvent control at $p < 0.05$ by Fisher's exact test. *Purity was more than 99.9%, and water was contained (< 0.1%).

Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP
Flag : Critical study for SIDS endpoint
07.05.2004 (26)

Type : Mouse lymphoma assay
System of testing : L5178Y Mouse Lymphoma cells
Test concentration : 60, 90, 135, 202, 301, 449, 670, 1000 µg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: not specified
Year : 1982
GLP : no
Test substance :

Result : Exposure to eight graded doses of the test substance in the presence and absence of metabolic activation increased the induction of forward mutations in L5178Y mouse lymphoma cells at the T/K locus. Sulfolane was considered to be mutagenic in this test system by the authors. However, there was no dose response and the survival percentage was not affected by increasing doses (see Table).
Test condition : A minimum of eight test compound doses with and without metabolic activation by Aroclor-induced rat liver microsomal fraction.

Appropriate negative, solvent and positive controls were included with each assay.

The maximum dose selected (1000 µg/mL) was based on solubility of the test material in the cell culture medium. The dose levels were determined by preliminary multi-dose ranging study.
Test substance : Chemical name: Sulfolane (CAS No. 126-33-0)
Purity: Assumed by author to be 100%
Supplied by: Phillips Petroleum Co.

Table: Summary of mouse lymphoma data

Treatment and dose level µg/mL	S-9	Percentage total survival	Mutation frequency ($x10^{-5}$)	Fold increase
Media	-	100.0	0.8	1.0
DMSO	-	94.2	0.8	-
EMS (620)	-	10.9	28.6	35.8
1000	-	72.8	2.6	3.3
670	-	60.4	4.6	5.8
449	-	74.0	4.4	5.5
301	-	71.3	5.0	6.3
202	-	56.1	6.0	7.5
135	-	71.2	4.1	5.1
90	-	75.6	4.3	5.4
60	-	60.1	4.8	6.0
Media	+	100.0	1.2	1.0
DMSO	+	93.7	1.9	-
MCA (3)	+	43.1	11.3	9.4
1000	+	55.1	3.4	2.8
670	+	59.5	5.9	4.9
449	+	62.3	4.5	3.8
301	+	51.1	4.1	3.4
202	+	54.5	5.5	4.6
135	+	34.5	8.9	7.4
90	+	68.3	1.9	1.6
60	+	37.5	8.3	6.9

Reliability : (2) valid with restrictions
Non-GLP study, not performed to guideline method

07.05.2004 (32)

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium: TA1535, TA1537, TA1538, TA98 and TA100 Escherichia coli: WP2 and WP2uvrA
Test concentration	:	31.25, 62.5, 125, 250, 500, 1000, 2000 or 4000 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Ames et al. (1975)
Year	:	1982
GLP	:	yes
Test substance	:	
Result	:	There was no increase in the reverse mutation frequency in any of the strains.
Test condition	:	The activities of the S9 mix and the sensitivities of the strains TA1538, TA98 and TA100 were monitored by treating cultures with a known positive control, benzo(a)pyrene. The sensitivity of TA1537 was monitored by the indirect mutagen Neutral Red; the sensitivities of the E.coli strains and TA1535 were monitored by testing with the direct acting mutagens 4-nitroquinoline-N-oxide or sodium azide respectively.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Study conducted to GLP, but not using Guideline method

07.05.2004 (47)

Type	:	Gene mutation in Saccharomyces cerevisiae
System of testing	:	Saccharomyces cerevisiae
Test concentration	:	0.01, 0.1, 0.5, 1.0, or 5.0 mg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Zimmerman (1977)
Year	:	1982
GLP	:	yes
Test substance	:	
Result	:	There was no consistent increase in the rate of mitotic gene conversion.
Test condition	:	Positive controls were 4-nitroquinoline-N-oxide and cyclophosphamide
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Study conducted to GLP, but not using Guideline method

07.05.2004 (47)

Type	:	Chromosomal aberration test
System of testing	:	Rat liver
Test concentration	:	250, 500, 1000 µg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: Dean et al. (1979)
Year	:	1982
GLP	:	yes
Test substance	:	

Result	:	There was no dose related increase in the incidence of chromosome damage in RL4 cells.
Test condition	:	After 24 hours the cultures were processed for chromosome analysis and where possible 100 cells analysed from each of three cultures per dose group.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Reliability	:	(2) valid with restrictions Study conducted to GLP, but not using Guideline method

07.05.2004 (47)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese Hamster Ovary Cells
Test concentration	:	0, 70, 210, 700, 2100 µg/ml in water and 6400 µg/ml
Cycotoxic concentr.	:	6400 µg/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: not specified
Year	:	1983
GLP	:	no
Test substance	:	
Method	:	The cells were exposed to five graded doses of sulfolane. The compound was added directly to the media at the highest dose and dissolved in glass distilled deionized water for the four lower doses.
Result	:	A statistically significant increase in the number of SCE's per chromosome was seen at the highest dose (p=0.003) in the absence of metabolic activation, but no significant increase was seen in the remaining dose levels and none showed a two-fold increase in SCE's. Under these conditions the chemical did not meet the criteria necessary to be considered positive and is therefore considered not to be mutagenic in this test system.
Test condition	:	No. of cells examined: 50 per group Positive controls: ethylmethanesulfonate (-S9) and cyclophosphamide (+S9)
Test substance	:	Chemical name: Sulfolane (CAS No. 126-33-0) Purity: Assumed by author to be 100% Supplied by: Phillips Petroleum Co.
Reliability	:	(2) valid with restrictions Study not conducted to GLP,or using Guideline method

07.05.2004 (34)

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, TA100
Test concentration	:	0, 642, 1925.9, 5777.8, 17333.3, 52000 µg/plate
Cycotoxic concentr.	:	>52000µg/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: not specified
Year	:	1982
GLP	:	no
Test substance	:	
Test condition	:	Replicates: 3 plates/dose Positive controls: Methylnitrosoguanidine, 2-nitrofluorene and 9-aminoacridine Metabolic activation via Aroclor-induced rat liver microsomal fraction.
Test substance	:	Chemical name: Sulfolane (CAS No. 126-33-0)

Purity: Assumed by author to be 100%
Supplied by: Phillips Petroleum Co.

Reliability : (2) valid with restrictions
Study not conducted to GLP, or using Guideline method

10.05.2004 (31)

Type : Ames test
System of testing : Salmonella Strains TA1535, TA1537, TA1538, TA98, TA100
Test concentration : 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method :
Year : 1982
GLP : yes
Test substance : no data

Source : Synthetic Chemicals Ltd, Wolverhampton
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

Type : Cytogenetic assay
System of testing : RL4 cells
Test concentration : 250, 500, 1000 µg/ml
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method :
Year : 1982
GLP : no data
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

Type : Escherichia coli reverse mutation assay
System of testing : WP2, WP2 uvrA
Test concentration : 31.25, 62.5, 125, 250, 500, 1000, 2000, 4000 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method :
Year : 1982
GLP : yes
Test substance : no data

Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature

17.06.2004 (16)

Type : Gene mutation in Saccharomyces cerevisiae
System of testing : Saccharomyces cerevisiae JDI
Test concentration : 0.01, 0.1, 0.5, 1.0, 5.0 mg/ml
Cycotoxic concentr. :
Metabolic activation : with and without

Result	:	negative
Method	:	
Year	:	1982
GLP	:	
Test substance	:	no data

Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

Type	:	
System of testing	:	Chinese hamster V79 Cell line
Test concentration	:	Dissolved in acetone (not exceeding 0.1%)
Cycotoxic concentr.	:	
Metabolic activation	:	no data
Result	:	negative
Method	:	
Year	:	1987
GLP	:	no data
Test substance	:	no data

Source	:	Synthetic Chemicals Ltd. Wolverhampton
Reliability	:	(4) not assignable Secondary literature

17.06.2004 (16)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Reproductive/developmental toxicity
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	Males: 49 days Females: 41 - 50 days from 14 days prior to mating to the 3rd day of lactation
Frequency of treatm.	:	Daily
Premating exposure period		
Male	:	14 days
Female	:	14 days
Duration of test	:	Males: 50 days. Females after day 4 of lactation
No. of generation studies	:	1
Doses	:	0, 60, 200, 700 mg/kg/day
Control group	:	yes, concurrent vehicle
Method	:	OECD Guide-line 421
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : NOAEL for reproductive performance: 700 mg/kg/day for males
200 mg/kg/day for females
NOAEL: Reproductive and developmental toxicity: 60 mg/kg/day

Mortality and day of death: One male and one female in the 700 mg/kg group died.

General toxic signs:
Males: Soiled fur, diarrhea, soft stool, supression of bodyweight gain and a decrease in food consumption were noted.
Females: Soiled fur, suppression in bodyweight gain during the pre-mating period, decreases in food consumption during the pre-mating and lactation periods, and an incraese in the relative ovary weight were noted.

Maternal data with dose level (with NOAEL value):
The number of oestrus cases decreased in the 700 mg/kg group. Four dams lost all of their pups during the lactation period in the 700 mg/kg group.

Pup data with dose level (with NOAEL value):
Birth index, live birth index, number of pups on days 0 and 4 of lactation, viablity index and bodyweights of both sexes on days 0 and 4 of lactation were decreased. The number of stillbirths increased in the 700 mg/kg group. Birth index and the number of pups on day 0 and 4 of lactation decreased in the 200 mg/kg group.

Test condition : TEST ANIMALS:
Number per sex, per dose: 12 per sex, per dose group
Weight at study initiation: 355 - 379g for males, 209 - 225g for females
Age at study initiation: 10 weeks for both sexes

TEST DESIGN:
The animals were sacrificed on day 4 of female lactation. Females that did not copulate were killed on the final day of the mating period. Females that did not give birth were killed on day 25 of pregnancy.

Vehicle: Water
Mating procedures: Male/females per cage = 1/1
Length of cohabitation = 14 days at most, until proof of copulation

Clinical observations performed and frequency:
Parent: general appearance twice a day
Pups: general appearance once a day after birth

Organs examined at necropsy:
Parent organ weight: testes, epididymis, ovaries
Parent microscopic: control and 700 mg/kg group - testes, epididymis, ovary
Pups: full macroscopic examination on all pups

Parameters assessed during the study:
Bodyweight and food consumption for males were determined twice a week, and for females bodyweight and food consumption were determined twice a week prior to mating and in principle, once a week for the gestation and lactation period. Oestrus cycle daily until successful copulation. No. of successful copulated pairs, copulation index, paring days until copulation, no. of pregnant females, fertility index, no. of corpora lutea, no. of implantation sites, implantation index, no. of living pregnant females, no of

preganant females with parturition, gestation length, no. of pregnant females with live pups on day 0, gestation index, no. of pregnant females with live pups on day 4, delivery index, no. of pups alive on day 0 of lactation, live birth index, sex ratio, no. of pups alive on day 4 of lactation, viability index, bodyweight of live pups (on day 0 and 4).

Table 1: Organ weight of male rats

Dose (mg/kg)	0	60	200	700
Number of males	12	12	12	11
Body Weight (g)	504.2±26.3	505.9±25.7	505.8±14.9	454.3±22.2**
Testes (g)	3.118±0.720	3.287±0.182	3.225±0.750	2.961±0.808
(g%)	0.619±0.143	0.652±0.054	±0.153	0.665±0.187
Epididymides (g)	1.172±0.226	1.230±0.083	1.211±0.222	1.087±0.236
(g%)	0.233±0.048	0.243±0.020	0.241±0.045	0.241±0.052

** Significantly different from control (p<0.01)

Table 2: Histopathological examination of male rats

Dose (mg/kg)	0						200						700					
Incidence & grade	N	A	±	+	2+	3+	N	A	±	+	2+	3+	N	A	±	+	2+	3+
Testis	[12]						[1]						[11]					
Atrophy, seminiferous tubule	10	2	0	1	0	1	0	1	0	0	0	1	9	2	0	1	0	1
Proliferation, Leydig's cell	11	1	1	0	0	0	0	1	0	1	0	0	10	1	0	1	0	0
Epididymides	[12]						[1]						[11]					
Decrease, sperm	11	1	0	0	0	1	0	1	0	0	0	1	10	1	0	0	0	1
Vacuolization, duct	11	1	0	1	0	0	1	0					10	1	0	1	0	0
Spermatic granuloma	12	0					1	0					10	1	0	1	0	0

Grade of histopathologic finding: ± slight, + mild, 2+ modetare, 3+ marked.
N: No abnormality detected
A: Abnormality detected
[ ] Number of males examined

Table 3: Organ weight of female rats

Dose (mg/kg)	0	60	200	700
Number of females	12	12	12	11
Body Weight (g)	289.0±21.3	290.3±19.2	284.0±15.0	268.3±14.2*
Ovaries (mg)	94.79±11.71	95.51±11.57	98.39±10.42	108.63±17.99
(mg%)	32.90±4.36	33.04±4.62	34.66±3.33	40.45±5.92**

* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)

Table 4: Number of Estrous cases and reproductive performance of male and female rats

Dose (mg/kg)	0	60	200	700
Number of females	12	12	12	12

Number of estrous cases before mating (14 days)	3.3±0.5	3.3±0.5	3.2±0.4	2.2±0.9**
Number of males	12	12	12	11
Number of males with successful copulation	12	12	12	12
Copulation index (%)[(a)]	100.0	100.0	100.0	90.9
Number of females	12	12	12	12
Number of females with successful copulation	12	12	12	11
Copulation index (%)[(b)]	100.0	100.0	100.0	91.7
Number of conceiving days	2.3±1.2	2.2±1.2	2.3±1.4	3.5±3.6
Number of pregnant females	11	12	10	10
Fertility index (%)[(c)]	91.7	100.0	83.3	90.9
Number o fpregnant females with live pups	11	12	10	10

** Significantly different from control (p<0.01)
(a) (number of males with successful copulation/number of males)x100
(b) (number of females with successful copulation/number of females)x100
(c) (number of pregnant females/number of females with successful copulation)x100

Table 5: Observation of pups (F1)

Dose (mg/kg)	0	60	200	700
Number of dams	11	12	10	10
Length of gestation (days)	22.18±0.40	22.08±0.29	22.00±0.00	22.00±0.00
Corpora lutea	16.4±1.3	16.8±1.4	17.7±2.2	17.0±3.3
Implantation scars	15.5±1.2	15.7±1.9	15.6±1.8	15.8±3.2
Implantation index (%) [(a)]	94.5±4.1	93.4±7.1	884±4.7	92.8±6.3
Gestation index (5) [(b)]	100.0	100.0	100.0	100.0
Pups born	15.2±1.7	15.2±1.7	14.3±1.8	14.9±3.4
Delivery index (%) [(c)]	98.1±4.5	96.9±4.0	91.8±4.1*	94.0±6.7
Live pups born	14.9±1.9	15.0±1.9	14.1±1.6	11.3±4.7
Sex ratio at birth [(d)]	0.98±0.67	1.12±0.55	1.73±1.09	0.78±0.33
Birth index (%) [(e)]	96.3±6.5	95.8±4.8	90.5±5.1*	71.6±26.2**
Dead pups on day 0 of lactation	0.3±0.5	0.2±0.4	0.2±0.4	3.6±4.4**
Live birth index (%) [(f)]	98.1±3.3	98.8±2.8	98.7±2.8	75.9±26.2**
Live pups on day 4 of lactation	14.8±1.8	15.0±1.9	13.7±1.3	4.0±5.6** (9)
Viability index (%) [(g)]	99.5±1.8	100.0±0.0	97.3±3.5	29.2±40.4** (9)
External anomalies (%) [(h)]	0.7±2.4	0.0±0.0	0.0±0.0	0.0±0.0
Anury (%)	0.7±2.4	0.0±0.0	0.0±0.0	0.0±0.0
Body weight of pups (g)				
Male Day 0	6.64±0.31	6.15±0.42*	6.22±0.38	5.44±0.52**
4	9.92±0.75	9.63±1.16	9.57±1.03	6.63±1.41** (4)
Female Day 0	6.20±0.30	5.88±0.33	5.85±0.44	4.93±0.46**
4	9.25±0.84	9.19±0.96	9.18±1.31	5.68±1.37** (5)

Each value shows mean±SD per dam
Figures in parentheses indicate number of dams
* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)
(a) (number of implantation scars/number of corpora lutea)x100
(b) (number of dams with live pups/number of pregnant dams)x100
(c) (number of pups born/number of implantation scars)x100
(d) number of male pups/number of female pups
(e) (number of live pups born/number of implantation scars)x100
(f) (number of live pups born/number of pups born)x100
(g) (number of live pups on day 4/number of live pups born)x100
(h) (number of pups with external anomalies/number of live pups born)x100

Conclusion : The NOAEL for maternal toxicity was 200 mg/kg.
The NOAEL for reproductive and development toxicity was 60 mg/kg/day
Reliability : (1) valid without restriction
Well conducted study performed to standard guidelines and under GLP
10.05.2004 (27)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : mouse
Sex : female
Strain :
Route of admin. : oral unspecified
Exposure period : no data
Frequency of treatm. :
Duration of test :
Doses : 93, 280, 840 mg/kg
Control group : no data specified
Method :
Year : 1987
GLP : no data
Test substance : no data

Remark : Skeletal changes at 850 mg/kg.
No effects at 93 and 280 mg/kg.
Source : Synthetic Chemicals Ltd. Wolverhampton
Reliability : (4) not assignable
Secondary literature
17.06.2004 (16)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Behavioural Effects
Study descr. in chapter :

Type : other: behavioural and autonomic thermoregulation
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : ip
No. of animals : 10
Vehicle : physiol. saline
Exposure period : 8 hour(s)
Frequency of treatm. : once
Doses : 800 mg/kg
Control group : yes, concurrent vehicle
Observation period : 8 hours
Result :
Method : other: not specified
Year : 1985
GLP : no data
Test substance :

Result : At ambient temperatures sulfolane caused a signficant

		inhibition in metabolic rate and reduction in colonic temperature. Metabolic rate tended to recover approximately 4 hrs after injection. Colonic temperature recovered but was still significantly reduced 8 hrs post-injection. Tail skin temperature was unaffected.
Test substance	:	Chemical Name: Sulfolane (CAS No. 126-33-0)
Conclusion	:	Since sulfolane toxicity appears to be greater with increased tissue temperature, the sulfolane induced hypothemria may enhance survival of the rat following exposure to toxic levels of sulfolane.

10.05.2004 (13)

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

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