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April 6, 2004

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Administrator Michael O. Leavitt
U.S. Environmental Protection Agency
PO BOX 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know HPV Consortium
Re: 2,5-dihydrothiophene 1,1-dioxide (Sulfolene)

Dear Administrator Leavitt:

Chevron Phillips Chemical Company LP is pleased to submit the 2-5-dihydrothiophene 1,1-dioxide (Sulfolene; CASN 77-79-2) Test Plan.

This submission is also being sent, via email, to the individuals listed below and opt.ncic@epa.gov; chem.rtk@epa.gov .

Sincerely,

Vicente Santa Cruz, Ph.D.

Enclosures:
Test Plan and IUCLID data sets on CASN 77-79-2 and CASN 126-33-0

Cc: R. Hefter, USEPA
O. Hernandez, USEPA

High Production Volume (HPV) Challenge Program

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2,5-dihydrothiophene 1,1-dioxide (Sulfolene)
CAS Number 77-79-2

Chevron Phillips Chemical Company LP
10001 Six Pines Drive
The Woodlands, Texas 77380

March 2004

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ABBREVIATIONS

BCF = predicted bioconcentration factor
cm^3 = cubic centimeter
CPChem = Chevron Phillips Chemical Company LP
EC = Commission of the European Communities
HEW = (United States) Department of Health, Education, and Welfare
hPa = hectopascal
HPV = High Production Volume
ICCA = International Council of Chemical Associations
IUCLID = International Uniform Chemical Information Dataset
IUPAC = International Union of Pure & Applied Chemistry
Koc = organic carbon partition coefficients
LC_{50} = lethal concentration (to 50% of animals dosed)
LD_{50} = lethal dose (to 50% of animals dosed)
LOAELs = lowest observed adverse effect levels
mg/kg = milligrams per kilogram
mg/L= milligrams per liter
NOAELs = no observed adverse effect levels
OECD = Organisation for Economic Cooperation and Development
SIDS = Screening Information Data Set
Sulfolane = tetrahydrothiophene 1,1-dioxide
Sulfolene = 2,5-dihydrothiophene 1,1-dioxide
USEPA = United States Environmental Protection Agency
µg = micrograms

I. EXECUTIVE SUMMARY

Chevron Phillips Chemical Company LP (CPChem) is committed to fulfilling the High Production Volume (HPV) commitments it made under the United States Environmental Protection Agency (USEPA) HPV Challenge Program on February 14, 2001. As part of this commitment, CPChem has volunteered to assess the health and environmental hazards, including selected physicochemical characteristics of 2,5-dihydrothiophene 1,1-dioxide (CASN 77-79-2), commonly known and referred to hereafter as Sulfolene. Sulfolene is a clear, solid, odorous organosulfur compound (C4H6O2S) used as a specialty solvent in petroleum refining and as a chemical intermediate in the production of tetrahydrothiophene 1,1-dioxide (Sulfolane).

CPChem has identified data from company proprietary files, peer-reviewed literature, and/or calculated endpoints using widely accepted computer modeling programs. In fulfillment with USEPA guidance for use of read-across data (USEPA, 1999b), CPChem proposes the use of surrogate data from Sulfolane (CASN 126-33-0), a close structural analogue to Sulfolene, to fill Screening Information Data Set (SIDS) endpoint data gaps and provide additional support in our understanding of health and environmental hazards for Sulfolene and Sulfolane. In addition, Sulfolane is currently being sponsored by Japan and CPChem through the International Council of Chemical Associations (ICCA) HPV Program. Both Sulfolane and Sulfolene have a relatively narrow range of physicochemical parameters and are composed of similar function groups. Thus, these two substances are expected to demonstrate similar environmental fate profiles and ecotoxicity as well as similar mammalian toxicity. Metabolism studies in rats show that Sulfolane is metabolized via ring hydroxylation into 3-hydroxytetrahydrothiophene-1:1-dioxide (Roberts and Warwick, 1961), which is consistent with expected biotic and/or abiotic hydrolysis of Sulfolene, and supports that these analogs have a common mode of action.

Physicochemical endpoints for Sulfolene are fulfilled by using existing measured data or data calculated by the EPIWIN© computer model. No additional testing is proposed for this program. A review of the existing data for Sulfolene and Sulfolane shows that sufficient data are available to characterize environmental fate and aquatic toxicity. An estimation from a Level III fugacity model predicts that these substances will likely partition to soil and water. Ready biodegradation testing showed that both Sulfolene and Sulfolane are not readily biodegradable and have very low predicted bioconcentration factors; organic carbon partition coefficients suggest similar fate profiles in the environment and no bioaccumulation hazard for either Sulfolene or Sulfolane. Acute fish, daphnid, and algal data indicated that Sulfolene is relatively nontoxic to aquatic organisms. Fish appear to be the most sensitive species with a lethal concentration to 50% of dosed organisms (LC_{50}) of 940 milligrams per liter (mg/L). No additional testing is proposed for environmental fate and ecotoxicity.

The considerable existing mammalian toxicity information for Sulfolene and Sulfolane demonstrates that these substances share a similar order of toxicity, regardless of the additional double bond in Sulfolene. Mammalian toxicity data demonstrates a low order

of toxicity via oral, dermal, and inhalation routes of exposure. Genotoxicity data exist for both Sulfolene and Sulfolane, and indicate that genotoxicity is not expected. Repeated dose toxicity testing on both Sulfolene and Sulfolane showed similar results in both rats and mice, and no further repeated dose toxicity testing is required. Sulfolene has not been tested for reproductive and developmental toxicity, but, due to its close structural similarity to Sulfolane, it would be expected to be of a similar order of magnitude as Sulfolane. Given that Sulfolane is characterized for this endpoint and Sulfolene is expected to produce results of the same order of magnitude, Sulfolane can be used as a structural surrogate for Sulfolene, and no further Sulfolene testing is warranted for this endpoint.

All HPV endpoints have been satisfied for the USEPA HPV Challenge Program. Table 1 summarizes the available data for Sulfolene and Sulfolane. No further testing is proposed for the USEPA HPV Challenge Program.

Table 1. Matrix of Available and Adequate Data on Sulfolene and Sulfolane

Test	Sulfolene Y/N (Klimisch Score)	Sulfolane Y/N (Klimisch Score)	Testing Planned? Y/N
Physical and Chemical Data			
Melting Point	Y (2)	N	N
Boiling Point	Y (2)	Y (1)	N
Vapor Pressure	Y (2)	Y (1)	N
Partition Coefficient	Y (1)	N	N
Water Solubility	Y (2)	N	N
Environmental Fate and Pathways			
Photodegradation	Y (2)	Y (2)	N
Stability in Water (Hydrolysis)	N	Y (1)	N
Transport/Distribution	Y (2)	Y (1)	N
Biodegradation	Y (1)	Y (1)	N
Ecotoxicity			
Acute/Prolonged Toxicity to Fish	Y (1)	Y (1)	N
Acute Toxicity to Aquatic Invertebrates (*Daphnia*)	Y (1)	Y (1)	N
Acute Toxicity to Aquatic Plants (Algae)	Y (1)	Y (1)	N
Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)	N	Y (1)	N
Toxicity			
Acute Toxicity (Oral)	Y (1)	Y (1)	N
Acute Toxicity (Inhalation)	Y (1)	N (4)	N
Repeated Dose	Y (2)	Y (1)	N
GeneticToxicity *in vitro* – Gene Mutation	Y (1)	Y (1)	N
Genetic Toxicity *in vitro* – Chromosomal Aberration	Y (2)	Y (1)	N
Reproductive Toxicity	N	Y (1)*	N
Developmental Toxicity	N	Y (1)*	N

* Reproduction/Developmental Toxicity Screen (OECD 421) was completed for Sulfolane.

***NOTE*:**
The data used to characterize the Organisation for Economic Cooperation and Development (OECD) SIDS endpoints for substances in this test plan were identified either in company proprietary files, the peer-reviewed literature, and/or calculated using widely accepted computer modeling programs. Sulfolane was used for read-across as defined by the USEPA (1999b). All data were evaluated for study reliability in accordance with criteria outlined by the USEPA (1999a). Only studies that met the reliability criteria of "1" (reliable without restrictions) or "2" (reliable with restrictions) were used to fulfill OECD SIDS endpoints. Additional data for Sulfolene and Sulfolane are also included in the IUCLID (International Uniform Chemical Information Dataset), attached in Appendices I and II. A more detailed discussion of the data quality and reliability assessment process used in developing this test plan is provided in Appendix III.

II. GENERAL SUBSTANCE INFORMATION

Sulfolene (CAS Number 77-79-2) is a commercially important product, with unique physical and chemical properties, that is used as an industrial solvent and isolated intermediate for the production of Sulfolane (CAS Number 126-33-0). Sulfolene is also used as a chemical intermediate in the production of transmission fluids.

Sulfolene is produced by the reaction of sulfur dioxide and 1,3-butadiene, and has a total production volume in the US of approximately 2 million pounds per year. Sulfolene is used commercially to remove benzene, toluene, and xylene from oil refinery streams and petroleum fuels, as well as to remove carbon dioxide from closed environments or scrubbing acids from various gas streams.

A majority of Sulfolene is subsequently hydrogenated to Sulfolane, another important solvent and close structural surrogate. Sulfolane is very well characterized from a physical/chemical, environmental, and human health point of view due to its HPV sponsorship by the ICCA and its numerous commercial applications, which include: selective solvents for liquid-vapor and aromatic hydrocarbon extractions; polymerization solvents; plasticizers, fractionation of wood tars, tall oil, and other fatty acids; textile finishing; curing agents for epoxy resins; and medicinal applications (patented and used as a vehicle for an injectable form of the drug heparin, as well as being developed as an encapsulated material for oral administration) (CPChem Internal Document, not dated; Budavari, 1989 in HSDB, 2003; Hawley, 1977 in HSDB, 2003; and Sax and Lewis, 1987 in HSDB, 2003).

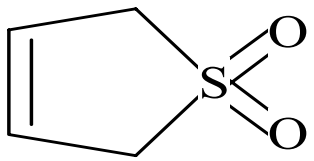
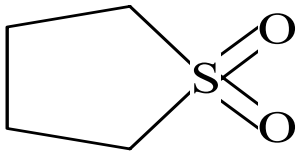
III. STRUCTURAL SURROGATE DISCUSSION

For Sulfolene, a substantial amount of data exists to characterize most of the OECD SIDS hazard endpoints and to meet the requirements of the USEPA HPV Challenge Program. However, Sulfolane also serves as a close structural surrogate to Sulfolene and adds to

the weight of evidence in characterization of many Sulfolene physical/chemical, environmental, and human health-related endpoints.

As is shown in Figure 1, these close structural analogs differ only in the presence of a carbon-carbon double bond versus a single bond in the thiophene ring structures. Otherwise, they posses identical functional groups.

Figure 1. Structural Comparison of Sulfolene and Sulfolane

Sulfolene	Sulfolane
	
International Union of Pure & Applied Chemistry (IUPAC) Chemical Name: 2,5-dihydrothiophene 1,1-dioxide CAS Number = 77-79-2 Molecular Weight = 118.15 SMILES: O=S(=O)(CC=C1),C1	IUPAC Chemical Name: Tetrahydrothiophene 1,1-dioxide CAS Number = 126-33-0 Molecular Weight = 120.17 SMILES: O=S(=O)(CCC1),C1

Importantly, in both Sulfolene and Sulfolane the five member thiophene ring contains the strongly electron withdrawing sulphone functional group (i.e. R-SO_2), which essentially lowers the activation energy needed for chemical reactions to occur at carbon atoms three and four, on the opposite side of the thiophene ring. This explains the nucleophilic ring hydroxylation at carbon number three that was observed in Sulfolane metabolism studies in rats (Roberts and Warwick, 1961). The double bond of Sulfolene is also located across from the sulphone functional group, which makes these carbon atoms at the double bond an excellent site for nucleophilic attack. Therefore, both Sulfolene and Sulfolane are expected to react with reactive species in vapor phase such as ozone, OH radicals, and UV light. In aqueous conditions, both will also be subject to biotic and abiotic transformation reactions such as ring hydroxylation and hydrolysis of the double bond. This inherent reactivity of Sulfolene is consistent with one of its commercial uses, which is scavenging acids. It applies to both biotic and abiotic chemical reactions and helps explain the strong similarity of physical/chemical and environmental fate and toxicological properties, as described in the following sections.

IV. PHYSICOCHEMICAL PROPERTIES

The physical chemical data for Sulfolene provided in Table 2 were primarily obtained from well-established and scientifically accepted reference handbooks such as the Merck Index (O'Neil, 2001) the Industrial Solvents Handbook (Flick, 1985), and Hawley's Condensed Chemical Dictionary (Lewis, 2001), as well as EPIWIN-calculated values

(USEPA and Syracuse Research Corporation, 2000). These data show that Sulfolene and Sulfolane both are highly water soluble and have similar melting points. The double bond in Sulfolene is expected to impart a slightly higher boiling point and vapor pressure to Sulfolene versus Sulfolane.

Table 2. Measured (M) and Calculated (C) Physicochemical Properties

Physical and Chemical Data				
Test	**M/C**	**Sulfolene**	**M/C**	**Sulfolane**
Melting Point	M[5,7]	63 - 65.5 ºC	M[7]	27.4 – 27.8 ºC
	M[1]	64.5 ºC	M[1]	27.6 ºC
	C[2]	17.43 ºC	C[2]	16.29 ºC
Boiling Point	M[6]	Decomposes	M[7]	285 (bp_{760})
			M[1]	287.3 ºC
	C[2]	201.11ºC	C[2]	198.49ºC
Vapor Pressure			M[5]	14.53 (302 ºF); 21.55 (320 ºF); 85.23 (392 ºF); 115.1 (410 ºF); 421.4 (500 ºF)
			M[1]	.0062 mmHg (.0083 hPa)
	C[2]	VP: 0.132 mmHg (0.176 hPa)	C[2]	0.004 mmHg (0.0053 hPa)
Kow Partition Coefficient	M[8]	HPLC method < 1.0; Fragment addition Method = -0.8	M[1]	-0.77
	C[3]	-0.45	C[3]	-0.24
Water Solubility	M[5]	5.90 wt% at 25 ºC	M[7]	At 30º, miscible with water
	C[4]	2.879 x 10^5 mg/L	C[4]	1.0 x 10^6 mg/L

[1]EPIWIN v3.10; measured values from the EPIWIN experimental database.
[2]EPIWIN v3.10; calculated using MPBPWIN v1.40 (determined at 760 millimeter mercury [mmHg]).
[3]EPIWIN v3.10; calculated using KOWWIN v1.66.
[4]EPIWIN v3.10; calculated using WSKOW v1.40.
[5]Flick, 1985.
[6]Lewis, 2001.
[7]O'Neil, 2001.
[8]USEPA, 1991b.

Summary: Adequate data (i.e., Klimisch rating 1 and 2) are available for all endpoints; no additional testing is proposed for the USEPA HPV Challenge Program (see Table 2 and IUCLID documents).

V. EVALUATION OF ENVIRONMENTAL FATE DATA

Environmental fate data for Sulfolene and Sulfolane were either experimentally measured or estimated using EPIWIN, and are provided in Tables 3, 3a, and 3b. Overall, these substances are expected to be mobile if released to the environment, but will disappear based upon both biotic and abiotic degradation mechanisms and do not pose any bioaccumulation hazard (as described below).

Table 3. Measured (M) and Calculated (C) Results for Environmental Fate and Pathways

Environmental Fate and Pathways				
Test	**M/C**	**Sulfolene**	**M/C**	**Sulfolane**
Photodegradation & Atmospheric Oxidation:			C[4]	Indirect photodegradation – half-life of 9.7 hr
• Ozone Rate Constant	C[1]	20 x 10^{-17} cm^3/molecule-sec	C[1]	No Data
• Ozone Half Life	C[1]	1.375 Hrs. (at 7x 10^{11} mol/cm^3)		
• OH Rate Constant	C[1]	65.73 x 10^{-12} cm^3/molecule-sec	C[1]	13.2 x 10^{-12} cm^3/molecule-sec
• OH Half Life	C[1]	1.953 Hrs	C[1]	9.666 Hrs
Stability in Water (Hydrolysis)		No Data	M[4]	Nominal: ca. 1000 mg/L Degradation: no hydrolysis at pH 4, 7, and 9 at 50 ± 1 ºC for 5 days
Transport/ Distribution				
Fugacity		See model results below (Table 3a)		See model results below (Table 3b)
Estimated Koc:	C[2]	21.59	C[2]	21.59
Estimated BCF:	C[3]	3.162	C[3]	3.162
Biodegradation	M[5]	2% after 28 days 0% after 28 days	M[4]	10% after 14 days

[1]EPIWIN v3.10; calculated using AOP Program v1.40.
[2]EPIWIN v3.10; calculated using PCKOC Program v1.66.
[3]EPIWIN v3.10; calculated using BCF Program v2.14.
[4]Japan IUCLID, 2003.
[5]USEPA, 1991c.

Summary: The weight of evidence in this test plan supports that no further environmental fate testing is necessary to meet HPV SIDS endpoints relating to environmental fate. Adequate data (i.e., Klimsch rating 1 and 2) are available for all endpoints, and no additional testing is proposed for the USEPA HPV Challenge Program (See Table 3 and IUCLID documents).

A. Photodegradation-Atmospheric Oxidation

Values for Sulfolene photodegradation and atmospheric oxidation were calculated based upon chemical structures using EPIWIN and are shown in Table 3. A calculated half-life for Sulfolene of 1.375 hours and rate constant of 20 x 10^{-17} cubic centimeter ($cm^{3)}$/molecule-sec has been estimated for reaction with ozone. EPIWIN was unable to estimate this reaction for Sulfolane due to lack of the carbon/carbon double bond. Also for reaction with hydroxyradicals, a calculated half-life for Sulfolene of 1.953 hours and a rate constant of 65 x 10^{-12} cm^3/molecule-sec has been estimated using EPIWIN, compared to a calculated half-life for Sulfolane of 9.666 hours and a rate constant of 13.2 x 10^{-12} cm^3/molecule-sec.

Summary: These results show that Sulfolene may be slightly more reactive, but are sufficient for USEPA HPV Challenge Program purposes and no further testing is warranted.

B. Hydrolysis

Both Sulfolene and Sulfolane are highly water soluble and are stable in solution. EPIWIN was unable to estimate a hydrolysis rate for the functional groups in Sulfolene and Sulfolane. Standard hydrolysis as a function of pH studies of Sulfolane show these materials are stable in water in the pH range of 4-9. pH alone, under the conditions of the standard OECD 111 "Hydrolysis as a function of pH" test, is not sufficient to catalyze hydrolysis of the thiophene ring. Further hydrolytic stability as a function of pH testing will not provide new useful information.

Summary: These results show that Sulfolene is not expected to be unstable under the conditions of the standard hydrolysis as a function of pH test. Existing information is sufficient for USEPA HPV Challenge Program purposes and no further hydrolysis testing is warranted.

C. Chemical Transport and Distribution in the Environment (Fugacity Modeling)

Tables 3a and 3b summarize the Level III Fugacity results for Sulfolene and Sulfolane produced by EPIWIN.

Table 3a. EPIWIN Level III Fugacity Results for Sulfolene

Compartment	100% to air	100% to water	100% to soil	Equally to each compartment
Air	69.6%	0.006%	0.03%	0.2%
Water	16.7%	99.8%	21.7%	55.9%
Soil	13.7%	0.001%	78.2%	43.8%
Sediment	0.03%	0.167%	0.04%	0.1%

Table 3b. EPIWIN Level III Fugacity Results for Sulfolane

Compartment	100% to air	100% to water	100% to soil	Equally to each compartment
Air	71.1%	0.1%	0.401%	2.93%
Water	16.4%	99.7%	21.7%	54.5%
Soil	12.5%	0.018%	77.8%	42.5%
Sediment	0.03%	0.166%	0.04%	0.1%

Summary: Results from the Level III fugacity modeling indicate that releases to water would remain in water while releases to air and soil would partition to water and soil. These results also show that both compounds behave similarly in the environment and further fugacity modeling is not warranted.

D. Biodegradation and Bioaccumulation

Sulfolene has been tested in two Commission of the European Communities (EC)/OECD Ready Biodegradation tests, the Closed Bottle Test, and the Modified Sturm Test. The results are reliable without restrictions and fulfill the HPV SIDS endpoint for Sulfolene. The results are also in agreement with EPIWIN calculated results. Sulfolene should be inherently biodegradable under real world aerobic and anaerobic conditions. However, under the conservative conditions of the standard OECD ready tests, Sulfolene was shown not to be readily biodegradable.

For additional perspective, the EPIWIN predicted bioconcentration factor (BCF) and organic carbon partition coefficients (Koc) are identical and very low for both Sulfolene and Sulfolane, suggesting similar fate profiles in the environment and no bioaccumulation hazard.

Summary: Adequate data (i.e., Klimsch rating 1 and 2) are available for all endpoints, and no additional environmental fate testing is proposed for the USEPA HPV Challenge Program (See Table 3 and IUCLID documents).

VI. ECOTOXICITY DATA

Acute fish, daphnid, and algal endpoints for Sulfolene are fulfilled with valid study data. The studies were conducted consistent with relevant OECD and USEPA guidelines. As shown in Table 4, both Sulfolene and its structural surrogate Sulfolane are relatively

nontoxic to aquatic organisms, and it would have been appropriate to use one structure as a read-across for the other had quality experimental data not been available for Sulfolene.

Aquatic studies have been performed on fish, aquatic invertebrates, and algae; fish appear to be the most sensitive species (LC_{50} = 940 mg/L for Sulfolene and LC_{50} > 100 mg/L for Sulfolane). There is also a chronic study available on *Daphnia magna* for Sulfolane.

Table 4. Results for Ecotoxicity Endpoints

Ecotoxicity		
Test	**Sulfolene**	**Sulfolane**
Acute/Prolonged Toxicity to Fish	96 hr LC_{50} = 940 mg/L[1]	LC_{50} > 100 mg/L[2]
Acute Toxicity to Aquatic Invertebrates (*Daphnia*)	24 hr EC_{50} > 1000 mg/L 48 hr EC_{50} = 800 mg/L[1]	NOEC = 171 mg/L EC_{50} = 852 mg/L[2]
Acute Toxicity to Aquatic Plants (Algae)	EC_{50} (growth rate) > 1000 mg/L[1]	NOEC (biomass) = 171 mg/L; NOEC (growth rate) = 309 mg/L (24-48h); EC_{50} (biomass) = 500 mg/L; EC_{50} (growth rate) > 1000 mg/L[2]
Chronic Toxicity to Aquatic Invertebrates (*Daphnia magna* reproduction test)	No Data Available	NOEC = 25.0 mg/L LCEC = 50.0 mg/L EC_{50} = >100 mg/L LC_{50} = >100 mg/L[2]

[1]USEPA, 1991b.
[2]Japan IUCLID, 2003.

Summary: No additional testing is proposed for ecotoxicity for the USEPA HPV Challenge Program (see Table 4 and IUCLID document).

VII. MAMMALIAN TOXICITY

The considerable existing mammalian toxicity information for Sulfolene and Sulfolane demonstrates that these substances share a similar order of toxicity, regardless of the additional double bond in Sulfolene. Mammalian toxicity data demonstrates a low order of toxicity via oral, dermal, and inhalation routes of exposure. Genotoxicity data exist for both Sulfolene and Sulfolane and indicate that genotoxicity is not expected. Repeated dose toxicity testing on both Sulfolene and Sulfolane showed similar results in both rats and mice, and no further repeated dose toxicity testing is required. Sulfolene has not been tested for reproductive and developmental toxicity, but, due to its close structural similarity to Sulfolane, and their common physical, chemical, and toxicologicial profiles,

Sulfolene would be expected to be of a similar order of reproductive and developmental toxicity as Sulfolane. Given that Sulfolane is characterized for this endpoint and Sulfolene is expected to produce results of the same order of magnitude, Sulfolane reproductive and developmental toxicity data can be used for read across purposes for Sulfolene, and no further Sulfolene mammalian toxicity testing is warranted to meet OECD SIDS endpoints.

Table 5. Results for Mammalian Toxicity Endpoints

Mammalian Toxicity		
Test	**Sulfolene**	**Sulfolane**
Acute Oral	3006.5 mg/kg (m)[1]; 2547.3 mg/kg (f)[1] 2876.1 mg/kg (combined)[1]	2006 mg/kg bw (m)[2] 2130 mg/kg bw (f)[2]
Acute Inhalation	LC_{50}: greater than the saturated concentration in air at 25°C. No deaths reported in dose group.[3]	No Data Available
Repeated Dose	Lowest observed adverse effect levels (LOAELs) for weight decrease: •Rat: 562 mg/kg/day (m); 178 mg/kg/day (f)[4] •Mouse: >3160 mg/kg/day (m); 316 mg/kg/day (f)[4] LOAELs for mortality: •Rat: >562 mg/kg/day (m); 316 mg/kg/day (f)[4] •Mouse: 1000 mg/kg/day (m); 1000 mg/kg/day (f)[4] No observed adverse effect levels (NOAELs) for weight decrease: •Rat: 316 mg/kg/day (m); 100 mg/kg/day (f)[4] •Mouse: >3160 mg/kg/day (m); 178 mg/kg/day (f)[4] NOAELs for mortality: •Rat: >562 mg/kg/day (m); 178 mg/kg/day (f)[4] •Mouse: 562 mg/kg/day (m); 562 mg/kg/day (f)[4]	**A** - NOAEL – • 60 mg/kg/day (m); 200 mg/kg/day (f)[2] LOAEL – • 200 mg/kg/day (m) - histopathological changes in the kidney); 700 mg/kg/day (f) - suppression of body weight gain and food consumption, and slight increase of GPT value in blood chem.)[2] **B** - NOAEL – • 200 mg/kg/day (both)[2] LOAEL – • 700 mg/kg/day (both) - death, clinical toxic signs, suppression of body weight gain[2]
Genetic – Gene Mutation	**A** – negative both with and without metabolic activation[5]; **B** – exp. to five graded doses with and without metabolic activation did not increase the reversion to histidine protrophy of five strains of *Salmonella typhimurium*[6]	Cytotox - No toxicity up to 5,000 µg/plate in five strains; Genotoxic effects negative both with and without metabolic activation[2]

Mammalian Toxicity		
Test	**Sulfolene**	**Sulfolane**
Genetic – Chromosomal Aberration	Negative with and without metabolic activity[7]	Cytotoxic concentration – 50% growth inhibition not observed at any concentration; Genotoxic effects negative for clastogenicity and polyploidy both with and without metabolic activation[2]
Reproduction/ Developmental Screen	No Data Available	NOAEL (reproductive performance) 700 mg/kg/day (m); 200 mg/kg/day (f) NOAEL (pups toxicity) 60 mg/kg/day[2] LOAEL – No Data Available[2]

[1] Hazleton, 1982a.
[2] Japan IUCLID, 2003.
[3] USEPA, 1991a.
[4] US Department of Health, Education, and Welfare [HEW], 1978.
[5] Hazleton, 1982c.
[6] Hazleton, 1982d.
[7] Loveday et al., 1990.

Summary: Data are available for all Mammalian Toxicity endpoints, as provided in Table 5 and described below. No additional testing is proposed for the USEPA HPV Challenge Program.

A. Acute Toxicity

Acute toxicity studies show that Sulfolene is of low acute toxicity by both the oral and inhalation routes (oral LD_{50} = 2,876.1 mg/kg [combined male and female]; LC_{50} greater than the saturated concentration in air at 25°C) (see Table 5 and IUCLID document). In addition, internal CPChem study summaries for which a full study report could not be located (and therefore were "not assignable" from a data quality perspective) provided the following supporting data for Sulfolene (see Table 5a):

- acute dermal LD_{50} of 1,930.4 mg/kg in male rabbits and 1,891.8 mg/kg in female rabbits; and
- acute inhalation LC_{50} of 458 ppm in male rats and 418 ppm in female rats.

Importantly, the close structural analogue Sulfolane was also tested and, in general, shows similar if not higher acute toxicity than Sulfolene.

Table 5a. Supportive Data for Mammalian Toxicity

Supportive Data for Mammalian Toxicity		
Test	**Sulfolene**	**Sulfolane**
Acute Dermal	1930.4 mg/kg (m); 1891.8 mg/kg (f) (Internal CPChem summary only)	1900-3500 mg/kg (male rabbit, subcutaneous) (Andersen et al., 1976)
Acute Inhalation	458 ppm (m); 418 ppm (f) (Internal CPChem summary only)	>12000 mg/m^3 (4 hr) (rat) (Andersen et al., 1977)

Summary: These studies fulfill the HPV requirements for the acute toxicity endpoint; no additional testing is proposed for the USEPA HPV Challenge Program.

B. Repeated Dose Toxicity

Sulfolene has also been tested by the NCI Carcinogenesis Testing Program in a chronic bioassay for possible carcinogenicity (HEW, 1978). As part of that program, a subchronic repeated dose study was performed on both rats and mice to determine dosing ranges for the chronic bioassay. While this study design does not meet OECD and USEPA guidelines, it was conducted under an adequately described protocol, and the study is available for review. The NCI study reported an NOAEL for mortality of 562 mg/kg/day for male rats and 178 mg/kg/day for female rats. In addition, NOAELS for a mortality of 562 mg/kg/day in both male and female mice were reported. These results are similar to the repeated dose toxicity observed with the close structural surrogate Sulfolane, which was tested in accordance with both the Japan Technical Guidance for 28-day repeated dose toxicity testing and OECD Technical Guideline 421.

It should be noted that the Sulfolene Bioassay will also be used as surrogate data for the Sulfolane ICCA HPV program to further support that long-term carcinogenicty effects are unlikely for Sulfolane.

Summary: The weight of evidence in this test plan, including the experimentally measured repeated dose toxicity results in rats and mice that agree closely with the repeated dose toxicity results from the structural surrogate Sulfolane, is sufficient to meet this HPV endpoint. No further repeated dose toxicity testing is required (see Table 5 and IUCLID Document).

C. Genetic Toxicity/Mutagenicity

1. Gene Mutation

Gene mutation tests conducted on Sulfolene have consistently resulted in negative results using the Ames Test. Equivalent results were also obtained for Sulfolane.

2. Chromosomal Aberrations

In vitro chromosomal aberration test results were identified for Sulfolene (Loveday et al, 1990) which showed Sulfolene to be negative for the following categories of chromosomal aberrations:

- "simple," defined as a chromatid gap, break, fragment, and deletion or chromosome gap, break, or double minuets;
- "complex," defined as interstitial deletions, triradials, quadriradials, rings, and dicentric chromosomes; and
- "other," defined as pulverized chromosomes or cells with greater than 10 aberrations.

Summary: Several valid *in vitro* genetic toxicity/mutagenicity studies have been performed for Sulfolene, all of which show no mutagenic activity for Sulfolene. Similar data for Sulfolane likewise show a lack of mutagenic activity for these materials. Adequate data are available for this endpoint, and no additional testing is proposed for the USEPA HPV Challenge Program (see Table 5 and IUCLID document).

D. Reproductive/Developmental Toxicity

Sulfolane was also tested in rats using the reproduction/development screening test pursuant to OECD Technical Guideline 421, with a data quality assessment of Klimisch 1, valid without restriction. The NOAEL for Sulfolane in this study is of the same order of magnitude as the repeated dose study, with an NOAEL for reproductive performance of 700 mg/kg/day in male rats and 200 mg/kg/day in female rats. Also, Sulfolane had an NOAEL of 60 mg/kg/day for production of pups. This study found that the toxic effects for female parents and pups were effects on reproductive parameters such as decrease of the number of estrus cases and increase of dams losing all of their pups. With regard to the pups, toxicity presented as effects on developmental parameters, including the number of pups, viability index, stillbirth, and body weight. No significant effect was observed showing grossly visible abnormalities in the pups.

Sulfolene has not been tested for reproductive and developmental toxicity, but, due to its close structural similarity to Sulfolane, it would be expected to be of a similar order of magnitude as reproductive and developmental toxicity of Sulfolane.

Summary: Given that Sulfolane is characterized for this endpoint and is expected to produce results of the same order of magnitude, Sulfolane

reproductive and developmental toxicity data can be used for read across purposes for Sulfolene. No further Sulfolene testing is warranted to meet OECD SIDS endpoints.

VIII. "BEYOND SIDS" ENDPOINTS

Studies have been performed on Sulfolene to investigate skin and eye irritation, and skin sensitization potential. A carcinogenicity study was also performed that demonstrated no significant increases in tumors in either sex compared to control groups (see IUCLID Document).

IX. CONCLUSIONS

As summarized below, CPChem concludes that there are sufficient, reliable data on Sulfolene and its structural surrogate, Sulfolane, following a thorough review of company proprietary files, peer-reviewed literature, and/or calculated using widely accepted computer modeling programs. No additional testing is recommended for the USEPA HPV Challenge Program.

Adequate data (i.e., Klimisch rating 1 and 2) are available for all endpoints; no additional testing is proposed for the USEPA HPV Challenge Program (see Table 2 and IUCLID documents).

- **PHYSICOCHEMICAL DATA**. Physicochemical endpoints for Sulfolene are fulfilled by using existing measured data or data calculated by the EPIWIN computer model. No additional testing is proposed.

- **ENVIRONMENTAL FATE**. Sufficient data are available to characterize environmental fate for Sulfolene. An estimation from a Level III fugacity model predicts that both Sulfolene, as well as its structural surrogate Sulfolane, will likely partition to soil and water. Ready biodegradation testing showed that Sulfolene and Sulfolane are not readily biodegradable and have very low predicted bioconcentration factors. Organic carbon partition coefficients suggest similar fate profiles in the environment and no bioaccumulation hazard for either Sulfolene or Sulfolane. Sufficient data are available to fulfill the environmental fate endpoints for Sulfolene and no additional testing is proposed.

- **ACUTE AQUATIC TOXICITY**. Scientifically reliable acute aquatic toxicity data are available and indicate that Sulfolene is relatively nontoxic to aquatic organisms. Sufficient data are available to fulfill the acute aquatic toxicity endpoint for Sulfolene and no additional testing is proposed.

- **ACUTE MAMMALIAN TOXICITY**. Mammalian toxicity data demonstrates a low order of toxicity via oral, dermal, and inhalation routes of exposure. Sufficient data are available to fulfill the acute toxicity endpoint for Sulfolene and no additional testing is proposed.

- **GENETIC TOXICITY**. Genotoxicity data exist for both Sulfolene and Sulfolane and indicate that genotoxicity is not expected. Sufficient data are available to fulfill the genetic toxicity endpoint for Sulfolene and no additional testing is proposed.

- **REPEATED DOSE TOXICITY**. Repeated dose toxicity testing on both Sulfolene and Sulfolane showed similar results in both rats and mice, and no further repeated dose toxicity testing is required.

- **REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**. Sulfolene has not been tested for reproductive and developmental toxicity, but, due to its close structural similarity to Sulfolane, it would be expected to be of a similar order of magnitude as Sulfolane. Given that Sulfolane is characterized for this endpoint and is expected to produce results of the same order of magnitude, Sulfolane can be used as a structural surrogate for Sulfolene, and no further Sulfolene testing is warranted for this endpoint.

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Appendix III

DATA QUALITY ASSESSMENT

Available environmental, ecotoxicity, and mammalian toxicity studies were reviewed and assessed for reliability according to standards specified by Klimisch et al., (1997), as recommended by the USEPA (1999a) and the OECD (OECD, 2002). The following reliability classification (Klimisch rating) has been applied to each study assessed:

- *1 = Reliable without Restriction* – Includes studies that comply with USEPA- and/or OECD-accepted testing guidelines and were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented;

- *2 = Reliable with Restriction* – Includes studies that were conducted according to national/international testing guidance and are well documented. May include studies that were conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters that are well documented and scientifically valid but vary slightly from current testing guidance. Also included in this category were physical-chemical property data obtained from reference handbooks, as well as environmental endpoint values obtained from an accepted method of estimation (e.g., USEPA's EPIWIN estimation program);

- *3 = Not Reliable* – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or in which documentation is insufficient; and,

- *4 = Not Assignable* – This designation is used in this dossier for studies that appear scientifically valid but for which insufficient information is available to adequately judge robustness.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this dossier. Those key studies selected for inclusion are considered typical of the endpoint responses observed in other studies of a similar nature and design that were identified during our search of the literature.

201-15167B1

Appendix I

Sulfolene

I U C L I D

Data Set

RECEIVED
OPPT CBIC
04 APR 12 PM 12:49

Existing Chemical	:	ID: 77-79-2
CAS No.	:	77-79-2
EINECS Name	:	2,5-dihydrothiophene 1,1-dioxide
EINECS No.	:	201-059-7
Molecular Formula	:	C4H6O2S

Producer Related Part

Company	:	Chevron Phillips Chemical Company LP
Creation date	:	13.11.2003

Substance Related Part

Company	:	Chevron Phillips Chemical Company LP
Creation date	:	13.11.2003
Memo	:	
Printing date	:	23.12.2003
Revision date	:	
Date of last Update	:	23.12.2003
Number of Pages	:	47
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD AND COMPANY INFORMATION

Type	:	other
Name	:	Chevron Phillips Chemical Company LP
Partner	:	
Date	:	
Street	:	10001 Six Pines Drive
Town	:	77380 The Woodlands, Texas
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	

21.11.2003

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

1-thia-3-cyclopentene 1,1-dioxide
13.11.2003

3-Sulfolene
13.11.2003

butadiene sulfone
13.11.2003

Sulfolene
13.11.2003

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value : = 63 - 65.5 ° C
Sublimation :
Method : other: not reported
Year :
GLP : no data
Test substance : other TS

Source : The Merck Index (O'Neil, 2001, 13th ed.) and the Industrial Solvents Handbook (Flick, 1985, 3rd ed.)

Test substance : 2,5-dihydrothiophene 1,1-dioxide [CAS 77-79-2]

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (6) (21)

Value : = 17.4 ° C
Sublimation :
Method : other: EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Method : EPIWIN v 3.10 - Selected Melting Point, Mean Value.

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
25.11.2003 (25)

2.2 BOILING POINT

Value : = 201.1 ° C at
Decomposition :
Method : other: EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Method : EPIWIN v 3.10 - Adapted Stein and Brown Method

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
25.11.2003 (25)

Decomposition : yes
Method : other: not reported
Year :

GLP : no data
Test substance : other TS

Remark : This substance decomposes above melting point

Source : Industrial Solvents Handbook (Flick, 1985, 3rd ed.) and Hawley's Condensed Chemical Dictionary (Lewis, 2001, 14th ed.)

Test substance : 2,5-dihydrothiophene 1,1-dioxide [CAS 77-79-2]

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (6) (17)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .175986 hPa at 25° C
Decomposition :
Method other (calculated): EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Method : EPIWIN v 3.10 - Selected Vapor Pressure (Modified Grain Method) using a boiling point of 201.11 deg C and a melting point of 65 deg C.

Result : Selected Vapor Pressure = 0.132 mm Hg (at 25 deg C). When converted to hPa, Vapor Pressure = 0.175986 hPa.

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
25.11.2003 (25)

2.5 PARTITION COEFFICIENT

Log pow : = - 0.8
Method other (calculated): fragment contribution calculation method
Year : 1983
GLP : no
Test substance : other TS

Method : Calculation of log Pow using the structural data file MACCS. The log Pow value was calculated from chemical structure using the fragment-addition method of Hansch and Leo (1979). An IMLAC (or VT/100) console with a light pen system was used to run the structural data file MACCS. An associated programme (CLOG P. REV 2.1) connected with this structural file enables calculation of log Pow. The programme is limited in that it

cannot handle any ionic, inorganic or organometallic compounds. Also, two of the correction factors, i.e. ring cluster and intramolecular hydrogen bonding, cannot be perceived and calculated. Further details of the capabilities, design and structure of this programme are provided elsewhere (Chou and Jurs, 1979).

Remark : Results indicate a low hydrophobility and low potential for Sulfolene to accumulate from water into organisms. (author)

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: Acute Toxicity (Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum), and N-Octanol/Water Partition Coefficient. (Experiment Number 2733). Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test substance : Sulfolene obtained from Shell Chemicals U.K. Ltd. Sample contained 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (2) (7) (27)

Log pow : < 1
Method other (measured): reverse-phase HPLC
Year : 1983
GLP : no
Test substance : other TS

Method : Method described by Eadsforth (1982). The HPLC system used was a reverse-phase C18-coated silica gel column (Partisil ODS-3), 250 mm x 5 mm id, with a mobile phase of 3 volumes methanol and 1 volume water (final pH 6.7) at a flow rate of 1 ml/min. Samples (25 ul) of an approximate 1 mg/ml solution in the above mobile phase were injected and the emergence of the material determined using refractive index detection. From the retention time of the peak the log Pow value was determined.

Remark : Results indicate a low hydrophobility and low potential for Sulfolene to accumulate from water into organisms. (author)

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: Acute Toxicity (Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum), and N-Octanol/Water Partition Coefficient. (Experiment Number 2733). Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test substance : Sulfolene obtained from Shell Chemicals U.K. Ltd. Sample contained 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (5) (27)

Log pow : = - 0.45
Method other (calculated): EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Method : EPIWIN v 3.10 - Log Kow used by Water solubility estimates.

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2).

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
25.11.2003 (25)

20.11.2003

2.6.1 WATER SOLUBILITY

Value : = 5.9 wt% at 25 ° C
Qualitative :
Pka :
PH :
Method : other: measured, method not reported
Year :
GLP : no data
Test substance : other TS

Source : Industrial Solvents Handbook (Flick, 1985, 3rd ed.)

Test substance : 2,5-dihydrothiophene 1,1-dioxide [CAS 77-79-2]

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (6)

Value : = 287900 mg/l
Qualitative : very soluble (> 10000 mg/L)
Pka :
PH :
Method : other: EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2).

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
25.11.2003 (25)

13.11.2003

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Deg. Product	:	
Method	:	other (calculated): EPIWIN v 3.10
Year	:	2003
GLP	:	no
Test substance	:	other TS
Method	:	Calculated using EPIWIN v 3.10 (AOP Program v1.90).
Result	:	Ozone Rate Constant = 20 E-17 cm3/molecule-sec Ozone Half Life = 1.375 hrs (at 7E11 mol/cm3) OH Rate Constant = 65.725 E-12 cm3/molecule-sec OH Half Life = 1.953 Hrs (12-hr day; 1.5E6 OH/cm3)
Source	:	EPI Suite v 3.10
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.11.2003 (25)

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air - water - soil - sediment
Air (level I)	:	
Water (level I)	:	
Soil (level I)	:	
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	other: EPI Suite v 3.10
Year	:	2003
Method	:	Level III Fugacity Model (EPI Suites). The following physical properties were used as the model input parameters: Chem Name: Thiophene, 2,5-dihydro-, 1,1-dioxide Molecular Wt: 118.15 Henry's LC: 4.27E-006 atm-m3/mole (Henrywin program) Vapor Press: 0.133 mm Hg (Mpbpwin program) Log Kow: -0.45 (Kowwin program) Soil Koc: 0.145 (calc by model)
Result	:	Results are provided in the following format: Compartment / 100% to Air / 100% to Water / 100% to Soil / Equally to

Each Compartment

Air / 69.6% / 0.006% / 0.03% / 0.2%
Water / 16.7% / 99.8% / 21.7% / 55.9%
Soil / 13.7% / 0.001% / 78.2% / 43.8%
Sediment / 0.03% / 0.167% / 0.04% / 0.1%

Air: half life = 1.017 hr; emissions = 1000 kg/hr
Water: half life = 360 hr; emissions = 1000 kg/hr
Soil: half life = 360 hr; emissions = 1000 kg/hr
Sediment: half life = 1440 hr; emissions = 0 kg/hr

Persistence when distributed equally to each compartment = 257 hr
(Emissions [kg/hr] = 1000 to air, 1000 to water, 1000 to soil, and 0 to sediment)

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2).

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (25)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : other: Micro-organisms were obtained from Canterbury Sewage Works and prepared according to the prescribed methods for this test.
Concentration : 20mg/l related to Test substance
related to
Contact time : 28 day
Degradation : = 2 % after 28 day
Result : other: not readily biodegradable
Control substance : Benzoic acid, sodium salt
Kinetic : 5 day > 50 %
15 day > 75 %
Deg. Product : not measured
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year : 1984
GLP : no data
Test substance : other TS

Result : Only 2% of the theoretically possible carbon dioxide was evolved by 28 days. According to the OECD guideline 301, the test substance cannot be considered as readily biodegradable.

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: An Assessment of Ready Biodegradability. Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : Sulfolene was added to the test medium from a stock solution containing 1 g/l to give a final test concentration of 20 mg/l sulfolene. The test medium was dispensed into the Sturm vessels, inoculated and aerated with 60 ml/min of CO2-free air at 25 +/- 1 deg. C. The extent of biodegradation at 1, 4, 7, 12, 19, 22, 26, and 28 days was determined by titrating the total carbon dioxide released from the incubation. The medium was acidified on day 27 to release the total carbon dioxide by day 28.

INOCULUM:
Micro-organisms were obtained from Canterbury Sewage Works and prepared according to the prescribed methods for this test.

CONTROLS:
Positive Control: Sodium benzoate was used as a degradable substance to demonstrate the activity of the microbial inoculum.
Control: Mineral medium
Blank: Microbial inoculum

Test substance : Approximately 90% sulfolene (2,5-dihydrothiophene 1,1-dioxide) and 7% isopropyl alcohol

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (28)

Type : aerobic
Inoculum : other: Micro-organisms were obtained from Canterbury Sewage Works and prepared according to the prescribed methods for this test.
Concentration : 3mg/l related to Test substance
related to
Contact time : 28 day
Degradation : = 0 % after 28 day
Result : other: Not readily biodegradable
Control substance : Benzoic acid, sodium salt
Kinetic : 5 day > 60 **%**

Deg. Product :
Method : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year : 1984
GLP : no data
Test substance : other TS

Remark : Sulfolene did not inhibit microbial activity in the Closed Bottle test, although some inhibition of the growth of Pseudomonas fluorescens was found in a separate test, 50% inhibition was not achieved even at a concentration of 1000 mg/l sulfolene.

Result : According to the OECD guideline 301, the test substance cannot be considered as readily biodegradable

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: An Assessment of Ready Biodegradability. Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : TEST DESIGN
-Sulfolene was added to the test medium from a stock solution of 1 g/l to give a final test concentration of 3 mg/l sulfolene.
-The bottles were incubated at 20 +/- 1 deg C and the extent of biodegradation determined by measuring the oxygen concentration in the bottles at 5, 15, and 28 days.

INOCULUM/TEST ORGANISM: Micro-organisms were obtained from Canterbury Sewage Works and prepared according to the prescribed methods for this test.

CONTROLS
Positive control: Sodium Benzoate
Control: Mineral medium
Blank: Microbial inoculum

INTERMEDIATES/DEGRADATION PRODUCTS: Not identified.

Test substance : Approximately 90% sulfolene (2,5-dihydrohtiophene 1,1-dioxide) and 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (28)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

BCF : = 3.16
Elimination :
Method : other: calculated with EPIWIN v 3.10
Year : 2003
GLP : no
Test substance : other TS

Method : Calculated using EPIWIN v 3.10 (BCF Program v 2.14)

The following parameters were used:
Log Kow (estimated): -0.45
Log Kow (experimental): not available from database
Log Kow used by BCF estimates: -0.45

Correction Factors Not Used for Log Kow <1.

Calculated Koc using EPIWIN v 3.10 (PCKOC Program v 1.66)

Result : Estimated Log BCF = 0.500
BCF = 3.162

Estimated Log Koc = 1.3343
Estimated Koc = 21.59

Source : EPI Suite v 3.10

Test substance : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2).

Reliability : (2) valid with restrictions
26.11.2003 (25)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
LC50	:	= 940
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	other TS

Method : Comparable to OECD Guideline 203 and U.S. EPA Guideline 797.1400 (OPPTS 850.1075).

Result : Cumulative Mortality:
Conc. in mg/l / 24hr / 48hr / 72hr / 96hr
0 / 0 / 0 / 0 / 0
100 / 0 / 0 / 0 / 0
200 / 0 / 0 / 0 / 0
500 / 0 / 0 / 0 / 0
1000 / 1 / 4 / 6 / 6

-No concentration caused 100% mortality

Mortality of Controls: 0

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: Acute Toxicity (Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum), and N-Octanol/Water Partition Coefficient. (Experiment Number 2733). Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : TEST ORGANISMS
- Rainbow trout, S. gairdneri, Itchen Valley Trout Farm, Alresford, Hampshire
- Mean length 3.7 cm (3.3 - 4.1)
- Mean weight 0.45 g (0.27 - 0.64)
- Measurements from sample 10 fish
- Acclimated to test conditions >10 days before test
- Number of dead fish recorded at 24 hr intervals

DETAILS OF TEST
- Static with daily renewal of test solutions

DILUTION WATER SOURCE
- From laboratory mains supply
- From two pumping stations (Newnham and Wychling) controlled by the Mid Kent Water Company. Water is obtained from bore holes in the chalk of the North Downs.
- Chemical treatment prior to arrival: chlorination to 0.1 mg/l

IN LAB TREATMENT:
- Filtered to remove particles larger than 8 um, chlorine, and organic compounds
- Stainless steel heat exchange units used to adjust temperature
- Aerated for several hours prior to test to remove residual chlorine

DILUTION WATER CHEMISTRY
- Range 13/10/80 - 4/10/83 (n=16)
- pH: 7.1-7.8
- Alkalinity: 253-275
- Hardness: 259-300

VEHICLE/SOLVENT AND CONCENTRATIONS
- Stock solution of Sulfolene in distilled water
- Logarithmic series of 4 concentrations between 100 and 1000 mg/l

NOMINAL TEST CONCENTRATIONS:
100 mg/l
200 mg/l
500 mg/l
1000 mg/l

MEASURED CONCENTRATIONS: No data

STABILITY OF THE CHEMICAL SOLUTION: Infra-red spectrum test confirmed substance was Sulfolene after 3 years of storage. On this basis material was considered stable for the duration of the study.

EXPOSURE VESSEL TYPE
- 10 fish in each aquarium
- 10 liters of water

TEST WATER CHEMISTRY
- pH: 7.8-8.5 (Control, 7.6-7.8; Top Concentration, 7.9-8.1)
- Hardness: 250-270 mg/l as $CaCO_3$
- Dissolved oxygen: 9.6-10.3 mg/l

TEST TEMPERATURE RANGE
- Monitored at 4 hourly intervals by a computer controlled thermocouple system which outputs when temperature deviates more than 2 deg C from nominal
- Range: 13 - 17 deg C for all groups through test

PHYSICAL MEASUREMENTS
- Test temperature: 13 - 17 deg C
- Concentration of dissolved oxygen (mg/L)
-- Control (mg/L):
--- 0 hr = 10.2
--- 24 hr = 9.6/10.0
--- 48 hr = 9.9/10.3
--- 72 hr = 9.7/9.6
--- 96 hr = 9.9
-- Top dose concentration (mg/L):
--- 0 hr = 10.2
--- 24 hr = 9.8/10.1
--- 48 hr = 9.9/10.1
--- 72 hr = 9.9/9.6
--- 96 hr = 9.9

- pH
-- Control:
--- 0 hr = 7.8
--- 24 hr = 8.4/8.2
--- 48 hr = 8.2/8.2
--- 72 hr = 8.2/8.2
--- 96 hr = 8.2
-- Top dose concentration:
--- 0 hr = 8.0

--- 24 hr = 8.5/8.4
--- 48 hr = 8.3/8.3
--- 72 hr = 8.3/8.2
--- 96 hr = 8.2

STATISTICAL METHODS: 96 hr LC50 was estimated by graphical interpolation using log/probit graph paper.

CONTROLS: One aquarium received no Sulfolene and served as a control.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) supplied by Shell Chemicals U.K. Ltd. Sample contained ~ 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (27)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
EC50 : = 800
95% fiducial limits : = 690 - 940
EC50, 24 h : > 1000
Method : other
Year : 1983
GLP : no
Test substance : other TS

Method : Comparable to OECD 202 and EPA 797.1330 (OPPTS 850.1300).

Result : Number Immobilized
Conc in mg/L / 24hr / 48hr
0 mg/L / 0 / 0
0 mg/L / 0 / 0
0 mg/L / 0 / 0

50 mg/L / 0 / 0
50 mg/L / 0 / 0
50 mg/L / 0 / 0

100 mg/L / 0 / 0
100 mg/L / 0 / 0
100 mg/L / 0 / 0

200 mg/L / 0 / 0
200 mg/L / 0 / 0
200 mg/L / 0 / 0

500 mg/L / 0 / 1
500 mg/L / 0 / 2
500 mg/L / 0 / 0

1000 mg/L / 2 / 5
1000 mg/L / 1 / 9

1000 mg/L / 0 / 8

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: Acute Toxicity (Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum), and N-Octanol/Water Partition Coefficient. (Experiment Number 2733). Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : TEST ORGANISMS: Daphnia magna, less than 24 hrs old, were taken from a culture in STL derived from a strain obtained (via ICI Brixham Laboratory) from I.R.Ch.A., France.

STABILITY OF THE CHEMICAL SOLUTION: Infra-red spectrum test confirmed substance was Sulfolene after 3 years of storage. On this basis material was considered stable for the duration of the study.

TEST TEMPERATURE RANGE
- Monitored at 4 hourly intervals by a computer controlled thermocouple system which outputs when temperature deviates more than 2 deg C from nominal
- Range: 18-22 deg C for all groups throughout test

EXPOSURE VESSEL
- 100 ml water in 150 ml glass crystallizing dishes
- 10 test animals per dish

WATER
- Water used for culturing and testing was a reconstituted fresh water prepared by dissolving the following amounts of Analar grade salts in glass distilled deionised water:

$NaHCO_3$ 192 mg/l
$CaSO_4*2H_2O$ 120 mg/l
$MgSO_4$ 120 mg/l
KCl 8 mg/l

TEST DESIGN
- 3 replicates
- 10 individuals per replicate
- Concentrations: logarithmic series of concentration ranging from 50 to 1000 mg/l:

TEST CONCENTRATIONS:
50 mg/l
100 mg/l
200 mg/l
500 mg/l
1000 mg/l

MEASURED CONCENTRATIONS: No data

IMMOBILIZATION
- Counted and recorded at 24 hr and 48 hr
- D. magna were considered to be immobile if, when the contents of the dish were briefly stirred they did not swim during a 10 minute period of observation.

EXPOSURE PERIOD: 48hr

STATISTICAL METHODS: 48hr EC50 was calculated using probit analysis after log transformation of the concentrations.

CONTROLS: Three dishes served as controls and received no sulfolene.

WATER CHEMISTRY IN TEST
- pH: 7.9-8.0 (for control and top concentration)
- Hardness: 170 mg/l as CaCO3
- Dissolved oxygen: 8.8-9.0 mg/l (for control and top concentration)

PHYSICAL MEASUREMENTS
- Test temperature: 18 - 22 deg C
- Total hardness: 170 mg/l as CaCO3
- Concentration of dissolved oxygen (mg/l)
-- Control (mg/l):
--- 0 hr = 9.0
--- 48 hr = 8.8
-- Top dose concentration (mg/l):
--- 0 hr = 9.0
--- 48 hr = 8.8
- pH
-- Control:
--- 0 hr = 8.0
--- 48 hr = 7.9
-- Top dose concentration:
--- 0 hr = 8.0
--- 48 hr = 7.9

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) supplied by Shell Chemicals U.K. Ltd. Sample contained ~ 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (27)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 4 day
Unit : mg/l
Analytical monitoring : no data
EC50 : > 1000
Method : other
Year : 1983
GLP : no
Test substance : other TS

Method : Comparible to OECD 201 and EPA 797.1050 (OPPTS 850.5400).

Result : Growth of S. capricornutum cultures exposed to a range of concentrations of Sulfolene

Cell density at each flask at each measuring point:
(Dose Concentration / Day 2 cell concentration [in cells/ml x 10 EE6] / Day 4 cell concentration [in cells/ml x 10 EE6])
0 mg/L / 0.013 / 0.47
/ 0.013 / 0.62
/ 0.015 / 0.65
/ 0.013 / 0.57
/ 0.012 / 0.39
/ 0.013 / 0.56
10 mg/L / 0.013 / 0.76

20 mg/L / 0.013 / 0.60
50 mg/L / 0.013 / 0.61
100 mg/L / 0.010 / 0.46
200 mg/L / 0.015 / 0.72
500 mg/L / 0.016 / 0.76
1000 mg/L / 0.013 / 0.73

Cell Number of Day 4 as % Mean Control Cell Number Day 4:
10 mg/l: 139
20 mg/l: 110
50 mg/l: 112
100 mg/l: 84
200 mg/l: 132
500 mg/l: 141
1000 mg/l: 134

Source : TSCA Section 8 (D) Health and Safety Data Reporting, Shell Oil Company submission. Study title: Sulfolene: Acute Toxicity (Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum), and N-Octanol/Water Partition Coefficient. (Experiment Number 2733). Testing Facility: Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : TEST TEMPERATURE RANGE
- Monitored at 4 hourly intervals by a computer controlled thermocouple system which outputs when temperature deviates more than 2 deg C from nominal
- Range: 22-26 deg C for all groups through test

GROWTH/TEST MEDIUM
- A nutrient medium was prepared by dissolving Analar grade salts in glass-distilled deionised water. Nutrient concentrations were those described by Miller and Green (1978) except that boric acid was present at 105 g/l, and sodium bicarbonate was present at 50 mg/l.
- The medium (excluding sodium bicarbonate) was autoclaved at 1.0 kg/cm2 for 15 min. On cooling, 20 ml/l of a millipore-serilized solution of sodium bicarbonate (2.5 g/l) was added.

EXPOSURE VESSEL TYPE: Erlenmeyer flask containing 50 ml of culture medium.

TEST ORGANISM: S. capricornutum were taken from a axenic culture in STL derived from a strain (ATCC 22662) obtained from the American Type Culture Collection, Maryland, USA.

TEMPERATURE, pH, AND WATER HARDNESS DURING TEST
- Measured at beginning and end of test
- Temperature: 22 - 26 deg C
- pH
-- Control:
--- 0 hr = 7.7
--- 2 day = 7.6
--- 4 day = 7.8
-- Top concentration:
--- 0 hr = 8.1
--- 2 day = 8.0
--- 4 day = 7.9
- Hardness: 170 mg/l as $CaCO_3$

LIGHTING
- Constant illumination (~3000 lux)

TEST DESIGN
- 7 flasks containing Sulfolene in distilled water to give logarithmic concentrations from 10 to 1000 mg/l
- 6 control flasks
- S. capricornutum 500 cells/ml
- Incubated in a cooled, orbital incubator (100 cycles/min)
- Cell counts after 2 and 4 days using Coulter Counter

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) supplied by Shell Chemicals U.K. Ltd. Sample contained ~ 7% isopropyl alcohol.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (20) (27)

19.11.2003

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	55
Vehicle	:	no data
Value	:	= 2876.1 mg/kg bw
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	other TS

Method : Comparable to OECD 401

Result : VALUE:
LD50 mg/kg (95% confidence interval):
Female: 2547.3 (2146.4 - 3023.0)
Male: 3006.5 (2440.9 - 3703.2)
Combined: 2876.1 (2544.3 - 3251.2)

NUMBER OF DEATHS AT EACH DOSE INTERVAL:
1000 mg/kg: 0 males, 0 females
2000 mg/kg: 0 males, 0 females
2500 mg/kg: 3 females
3000 mg/kg: 2 males, 5 females
4000 mg/kg: 5 males, 4 females
5000 mg/kg: 5 males, 5 females

TIMES OF DEATH:
2500 mg/kg: 3 females (1 on day 1, 2 on day 7)
3000 mg/kg: 2 males (1 on day 2, 1 on day 4); 5 females (5 on day 1)
4000 mg/kg: 5 males (1 at hour 4, 4 on day 1); 4 females (2 at hour 4, 2 on day 1)
5000 mg/kg: 5 males (3 at hour 2, 2 on day 1); 5 females (2 at hour 2, 3 on day 1)

CLINICAL OBSERVATIONS
- Clinical observations were noted among all rats by one, two, or four hours post dose.
- Included: depression, slight depression, rough coat, urine stains, thinness, red stains on nose/eyes, soft feces, a hunched appearance, tremors, salivation, lacrimination, ataxia, prostration, labored respiration, and convulsions.
- All rats that survived to termination gained weight.
- All rats that died lost weight, with the exception of one that gained weight.
- No observable gross pathology was noted in rats surviving to termination.
- Alterations of the stomach were most consistent among the animals that died and included: distension of stomach and intestines, compound-like material, dark red material, thick brackish fluid, yellowish fluid, or reddish fluid in the stomach and/or intestines.
- Other findings in the lung and liver were considered incidental.

Source : Phillips Petroleum Company Acute Oral Toxicity Study in Rats - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : DOSE CONCENTRATIONS: 1000 mg/kg, 2000 mg/kg, 2500 mg/kg, 3000

mg/kg, 4000 mg/kg, 5000 mg/kg

ROUTE OF ADMINISTRATION: Gavage

TEST CONDITIONS:
- Age: young adult (200 - 300 grams).
- Two animals per cage.
- 12 hour light-dark cycle.
- Single dose of test material, animals fasted 18 to 24 hours prior to dosing.
- Group 1 dosed with 5000 mg/kg, results reported after 48 hours indicated need for additional groups to be added to determine LD50.
- 5 male and 5 female rats per dose concentration with the exception of only 5 females (no males) being dosed at 2500 mg/kg.
- Observation of Animals: Day of dosing - 1,2, and 4 hours; twice daily thereafter. Observations include nature, onset, severity, and duration of pharmacotoxic signs.
- Body Weights: Taken just prior to treatment (dosage volume for each rat is based on this weight), at death, and/or at seven and 14 days.
- Post dose observation period: 14 days
- At study termination:
-- Animals that Succumb: Necropsies were performed by appropriately trained personnel under procedures supervised by board-certified pathologist.
-- Sacrifice: At Day 14, all surviving animals were weighed, anesthetized, and exsanguinated. Necropsies were performed by appropriately trained personnel under procedures supervised by board-certified pathologist.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
25.11.2003 (8)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Species : rat
Strain : Wistar
Sex : male/female
Number of animals : 10
Vehicle : no data
Exposure time : 4 hour(s)
Method : other
Year : 1980
GLP : no data
Test substance : other TS

Method : Comparable to OECD 403.

Result : LC50: greater than the saturated concentration in air at 25°C. No deaths reported in dose group.

CLINICAL SIGNS: No clinical signs were reported for any dose animals.

NECROPSY FINDINGS
- Macroscopic: Four dose animals had lungs that appeared dark, pale, or patchy at necropsy.
- Microscopic: Focal intra-alveolar haemorrhage and slight alveolar collapse in two of the exposed animals. Evidence of this type was also seen in control rats, and absence of signs of pulmonary irritation lead to

conclusion that microscopic findings are not treatment induced.

Source : TSCA Section 8 (D) Health and Safety Data Reporting Shell Oil Company submission. Study report entitled "Toxicology of fine chemicals: The acute 4 h inhalation LC50 of sulfolene in rats. Testing facility was Sittingbourne Research Centre, Sittingbourne, Kent.

Test condition : Approximately 99% sulfolene (2,5-dihydrothiophene 1,1-dioxide) and 0.9% isopropyl alcohol

Test substance : NUMBER OF ANIMALS PER SEX PER DOSE GROUP:
5 male/5 female/dose
10 male/10 female/control

AGE: 8-9 weeks
WEIGHT: females 171-204 g, males 285-316 g

EXPOSURE CHAMBERS
- Test animals housed in two 7 litre tubular glass chambers fitted with stainless steel mesh carriers to accomodate five animals each.
- Test atmosphere supplied to each chamber at a minimum flow rate of 10 L/min.
- Chambers located in the fume cupboard together with the atmosphere generator.

ATMOSPHERE GENERATOR: Test atmosphere was generated using a modified version of the wick method.

VOLUME ADMINISTERED: 20 litres of clean dry air per minute passed through condenser tube packed with 80 g Sulfolene and an approximately equal amount of glass fractionation column packing material. Maintained at a temperature of 29.5°C by circulating water from a thermostatically controlled water bath, through the condenser jacket.

DOSE ATMOSPHERE ANALYSIS
- Air saturated
- Method for the continuous analysis of sulfolene/air mixtures was not available, evidence of saturation obtained from the tendency of condensation to occur in the cooler parts of the exposure apparatus.
- Test atmosphere was analysed for sulfur dioxide and isopropyl alcohol continuously throughout the exposure period using a MIRAN 1 infra-red gas analyser. The latter was calibrated in a closed recycle loop system into which known amounts of sulphur dioxide and isopropyl alcohol were introduced using a microlitre syringe. A separate check for the sulphur dioxide content of the test atmosphere was made using chemical indicator tubes.

EXPOSURE DURATION: 4 hours

CONTROL GROUP
- 10 male and 10 female rats were housed in hangimg stainless steel mesh cages throughout the duration of the experiment.
- Control group was used only for the determination of body weights and was not submitted for pathological examination.

POST DOSE OBSERVATION:
- Observed daily for toxic signs over 14 days following exposure.
- Body weights were recorded in the week prior to exposure and 14 days post exposure.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

20.11.2003 (26)

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
PDII	:	0
Result	:	not irritating
EC classification	:	not irritating
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	other TS

Method : Comparable to OECD 404 - Acute Dermal Irritation/Corrosion

Result : No erythema, edema, or other dermal effects were noted at 24 or 72 hours after administration of Sulfolene. The primary irritation score is calculated to be zero.

Source : Phillips Petroleum Company Primary Skin Irritation Study in Rabbits - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : Species: Rabbit
Strain: New Zealand White/ Dutchland
Number/Sex: Three adult males and three adult females
Housing: Housed individually
Environment: temperatures maintained at 70 +/- 4 deg F with a relative humidity of 40-60%. A 12 hour light-dark cycle was maintained.

Test Description:
- Six albino rabbits clipped free of hair
- Test material administered to one abraded site and one intact site on the back of each animal with the sites rotated over various areas of the clipped skin.
- Dose (0.5 g) applied undiluted (moistened with physiological saline before application)
- 0.5 g of solid test material introduced at each application site under a 1 inch to 1-1/2 inch square guaze patch, secured with transparant tape.
- Entire trunk of the animal wrapped with a non-absorbent binder and the animal was immobilized in a stock for 24 hours.
- After 24 hours exposure, patches were removed and the skin was wiped to remove any test substance still remaining.

Observation of Animals: The skin reactions were evaluated at 24 and 72 hours following the initial application of test material.

Scoring:
- Erythema and Eschar Formation:

No erythema ---> 0
Very slight erythema (barely perceptible) ---> 1
Well-defined erythema ---> 2
Moderate to severe erythema ---> 3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) ---> 4

- Edema Formation:
No edema ---> 0
Very slight edema ---> 1
Slight edema (edges of area well-defined by definite raising) ---> 2
Moderate edema (raised approximately 1 mm) ---> 3
Severe edema (raised more than 1 mm and extending beyond the area of exposure) ---> 4

The primary skin irritation score is calculated by dividing the sum (8 values) of the erythema and edema means at 24 and 72 hours for the abraded and intact skin sites by four.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity.
Reliability : (2) valid with restrictions
19.12.2003 (12)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : other: normal saline slurry
Dose : 100 other: mg
Exposure Time :
Comment : not rinsed
Number of animals : 6
Result : irritating
EC classification :
Method : other
Year : 1983
GLP : no data
Test substance : other TS

Result : The mean results are presented in the following format:
Observation / 1 hr / 24 hr / 48 hr / 72 hr / 4 days / 7 days

Cornea / 5.8 / 19.2 / 19.2 / 15.0 / 14.2 / 5.8

Iris / 1.7 / 4.2 / 4.2 / 2.5 / 1.7 / 1.7

Conjunctivae / 9.3 / 12.3 / 13.0 / 11.7 / 9.7 / 4.3

Total Score / 16.8 / 35.7 / 36.3 / 29.2 / 25.5 / 11.8

Corneal opacity, involving up to 100% of the cornea, was noted in three rabbits throughout the study and in three additional rabbits from twenty-four hours postinstillation to Day 4. Iritis was noted in five rabbits by one or twenty-four hours postinstillation. Conjunctival redness (vessels definitely injected above normal to diffuse beefy red), conjunctival chemosis (swelling above normal to swelling with lids about half closed), and conjunctival discharge (any amount above normal to moistening of the lids and hairs, and considerable area around the eye) were noted in all six rabbits. Phonation upon instillation of the test material was noted in one rabbit. Ocular irritation was present in all six rabbits at termination of the study.

Source : Phillips Petroleum Company Unwashed Primary Eye Irritation Study in Rabbits - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : Species: Rabbit
Strain: New Zealand White/ Dutchland
Number/Sex: Six young adult animals per group
Housing: Housed individually
Environment: temperatures maintained at 70 +/- 4 deg F with a relative humidity of 40-60%. A 12 hour light-dark cycle was maintained.

Test Description:
- The left eye of six albino rabbits were examined 24-72 hours prior to instillation of the test materials with fluorescein dye solution. Animals showing corneal damage were not used.
- Dose: 0.1 ml undiluted test material for liquids, 100 mg sample as a normal saline slurry for solids or pastes.
- Treated eyes were held closed for one second following instillation and were not washed.
- The untreated right eye of each rabbit served as a control.

Observation of Animals:
- Ocular reactions were evaluated at 24, 48, and 72 hours, and at 4 and 7 days after treatment.
- Scoring was done according to the Draize system of scoring.
- Fluorescein Staining was done after the reading at 24 hours. The eyes of the rabbits were examined with fluorscein dye solution. Any corneal damage was reconfirmed by examination with fluorescein dye solution at subsequent readings.
- The treated eye of each animal was re-examined at the termination of the study using the fluorescein dye solution to confirm the absence or presence of any corneal damage.
- Body weights were determined initially and terminally.
- The study was terminated on the seventh day, all rabbits were sacrificed with T-61.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity.
Reliability : (2) valid with restrictions
23.12.2003 (14)

Species : rabbit
Concentration : other: normal saline slurry
Dose : 100 other: mg
Exposure Time :
Comment :
Number of animals : 6
Result : irritating
EC classification :
Method : other
Year : 1983
GLP : no data
Test substance : other TS

Result : The mean results are presented in the following format:
Observation / 1 hr / 24 hr / 48 hr / 72 hr / 4 days / 7 days

Cornea / 20.0 / 4.2 / 5.8 / 3.3 / 3.3 / 1.7

Iris / 2.5 / 1.7 / 0.8 / 0.8 / 0.8 / 0.0

Conjunctivae / 11.7 / 7.7 / 8.3 / 4.7 / 3.3 / 2.7

Total Score / 34.2 / 13.5 / 15.0 / 8.8 / 7.5 / 4.3

Corneal opacity, involving up to 100% of the eye, was noted in all six rabbits by one hour postinstillation of Sulfolene. Corneal opacity persisted to a lesser degree in one rabbit each to twenty-four hours, seventy-two hours, and Day 4 and reoccurred in one rabbit at forty-eight hours to termination. Iritis was noted in five rabbits at varying intervals during the study. Conjunctival redness (vessels definitely injected above normal to diffuse beefy red), conjunctival chemosis (swelling above normal to obvious swelling with partial eversion of the lids), and conjunctival discharge (any amount above normal to moistening of the lids and hairs, and considerable area around the eye) were noted in all six rabbits. Phonation upon instillation of the test material was noted in one rabbit. Ocular irritation was present in five rabbits at termination of the study.

Source : Phillips Petroleum Company Washed Primary Eye Irritation Study in Rabbits - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : Species: Rabbit
Strain: New Zealand White/ Dutchland
Number/Sex: Six young adult animals per group
Housing: Housed individually
Environment: temperatures maintained at 70 +/- 4 deg F with a relative humidity of 40-60%. A 12 hour light-dark cycle was maintained.

Test Description:
- The left eye of six albino rabbits were examined 24-72 hours prior to instillation of the test materials with fluorescein dye solution. Animals showing corneal damage were not used.
- Dose: 0.1 ml undiluted test material for liquids, 100 mg sample as a normal saline slurry for solids or pastes.
- Dosed in the conjunctival sac of the left eye of each test animal.
- Treated eyes were held closed for four seconds following instillation and then the eyes were washed with 40 ml of tap water.
- The untreated right eye of each rabbit served as a control.

Observation of Animals:
- Ocular reactions were evaluated at 24, 48, and 72 hours, and at 4 and 7 days after treatment.
- Scoring was done according to the Draize system of scoring.
- Fluorescein Staining was done after the reading at 24 hours. The eyes of the rabbits were examined with fluorscein dye solution. Any corneal damage was reconfirmed by examination with fluorescein dye solution at subsequent readings.
- The treated eye of each animal was re-examined at the termination of the study using the fluorescein dye solution to confirm the absence or presence of any corneal damage.
- Body weights were determined initially and terminally.
- The study was terminated on the seventh day, all rabbits were sacrificed with T-61.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity.
Reliability : (2) valid with restrictions
23.12.2003 (15)

5.3 SENSITIZATION

Type : Guinea pig maximization test
Species : guinea pig

Concentration : Induction 40 % occlusive epicutaneous
Challenge 40 % occlusive epicutaneous

Number of animals : 40
Vehicle : other: acetone
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1982
GLP : no data
Test substance : other TS

Result : Mortality and Clinical Observations: No deaths occurred and all animals appeared normal throughout the study.

Dermal Responses:
- Guinea pigs treated with 0.1% DNCB in acetone during the challenge phase only exhibited no dermal irritation at 24, 48, or 72 hours. Animals exposed to the same challenge concentration of DNCB, following the induction with 0.25% 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. Comparison of these dermal responses indicate that the guinea pigs responded to hypersensitization when a known sensitizer was used.
- Guinea pigs receiving the test material, 40% sulfolene in acetone, during the challenge phase only and those animals exposed to the same challenge dose following the induction with 40% sulfolene in acetone and rest period exhibited no dermal irritation at 24, 48, or 72 hours.

Based on this reponse, sulfolene is not considered a dermal sensitizer in guinea pigs.

Source : Phillips Petroleum Company Dermal Sensitization Study in Guinea Pigs - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : Species: Guinea Pig
Strain: Hartley from Dutchland Laboratory Animals, Inc.
Number/Sex: 20 males and 20 females
Age at Initiation: Adult, 300-500 grams
Housing: Housed individually
Environment: temperatures maintained at 70 +/- 4 deg F with a relative humidity of 40-60%. A 12 hour light-dark cycle was maintained.

Test Description:
- Group 1 (Positive control) - 10 animals, induction and challenge with dinitrochlorobenzene (DNCB)
- Group 2 - 10 animals, induction and challenge with 40% sulfolene
- Group 3 (Positive control) - 10 animals, challenge only with DNCB
- Group 4 (Controls) - 10 animals, challenge only with 40% sulfolene

Determination of Induction and Challenge Levels:
- Number: 4
- Site per animal: 4
- Induction Phase: The upper left quadrant of the backs of the guinea pigs in the test group were clipped free of hair. The following day (Day 1), 0.5 ml of test solution was applied to the shaved area near the mid line of the back and a patch (3/4" x 1" Webril Appli-Pad) held in place with Dermiclear brand transparent tape was applied. Rubber daming was wrapped around each animal to secure the patch and each pig was placed in an individual restraining device. The patches were left in place for six hours.
---- Reapplication: once per week for three weeks.

---- Control Animals: no treatment
- Challenge Phase: Two weeks following the administration of the last induction patch, the lower left quadrant of the backs of both test and control animals was shaved. The following day, 0.5 ml of the highest non-irritating dose of the test compound was applied to the shaved back for a 4 to 6 hour exposure period as performed in the induction phase.
- Dipilation: The day following the challenge phase, the lower quadrant of each pig was dipilated with Zip by applying Zip to the area and allowing it to remain in contact with the skin for 20 to 30 minutes and then washing it off with tap water.

Observation:
- Twenty-four hours (three to five hours post dipilation), 48, and 72 hours folowing administration of the challenge dose.
- The challenge sites were scored for erythema and edema according to the system of Draize.
- After the evaluation of skin sites 72 hours following the challenge dose, all animals were sacrified with T-61.
- A skin section from the challenge site was fixed in 10% neutral buffered formalin, after the 72 hour challenge observation, for histopatholgic evaluation.

Interpretation: No reactions greater than 1 should be seen in any of the control animals. Any reaction of grade 2 or greater on test animals that is stronger than the most severe response elicited by the controls will be considered a positive response.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity.
Reliability : (2) valid with restrictions
23.12.2003 (9)

5.4 REPEATED DOSE TOXICITY

Species : rat
Sex : male/female
Strain : Osborne-Mendel
Route of admin. : gavage
Exposure period : 6 weeks
Frequency of treatment : 5 consecutive days per week for 6 weeks
Post obs. period : 2 weeks
Doses : 0 (corn oil control), 56, 100, 178, 316, 562 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL : = 100 - 316 mg/kg
LOAEL : = 178 - 562 mg/kg
Method : other
Year : 1978
GLP : no
Test substance : other TS

Method : Subchronic toxicity for National Cancer Institute Bioassay maximum tolerated dosages selection.

Result : ENDPOINTS EXAMINED: Mortality and weight changes.

LOAEL:
- Weight decrease: male rats = 562 mg/kg/day
- Weight decrease: female rats = 178 mg/kg/day
- Mortality: male rats = >562 mg/kg/day
- Mortality: female rats = 316 mg/kg/day

NOAEL:
- Weight decrease: male rats = 316 mg/kg/day
- Weight decrease: female rats = 100 mg/kg/day
- Mortality: male rats = >562 mg/kg/day
- Mortality: female rats = 178 mg/kg/day

ADDITIONAL REMARKS: The only deaths observed among treated rats were 2 females, one receiving 316 mg/kg/d and the other receiving 562 mg/kg/d. Mean body weight depression was 17% in males treated with 562 mg/kg/d and 18% in females treated with 178 mg/kg/d. The high dosages of 3-sulfolene selected for use in the chronic bioassay were 560 mg/kg/day for male rats and 200 mg/kg/day for female rats.

Source : National Cancer Institute Carcinogenesis Technical Report Series No. 102, 1978.

Test condition : Test performed as a range-finder to determine maximum tolerated dose for a long-term carcinogenicity assay.

ANIMAL MAINTENANCE
- Rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors in temperature- and humidity-controlled rooms.
- Temperature range was 20 - 24 deg C and the relative humidity was maintained between 45 and 55 percent.
- The air conditioning system in the laboratory provided filtered air at a rate of 12 to 15 complete changes of room air per hour.
- Fluorescent lighting was provided on a 12-hour-daily cycle.

NUMBER OF ANIMALS DOSED: Six groups, each consisting of five males and five females.

GASTRIC INTUBATION
- 3-Sulfolene mixed with corn oil was introduced by gavage to five of the six rat groups at dosages of 56, 100, 178, 316, and 562 mg/kg/day.
- The sixth group served as a control, receiving only the corn oil by gavage.
-Intubation was performed for five consecutive days per week for 6 weeks on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose.
- All animals of one sex within a treated group received the same dose.

OBSERVATION PERIOD: Two weeks after termination of dosing to detect any delayed toxicity.

Test substance : 3-Sulfolene purchased from Phillips Petroleum Company. Chemical analysis performed by Hazleton Laboratories America, Inc., Vienna, Virginia. The purity of the test chemical was indicated to be approximately 92 percent.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (24)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 6 weeks
Frequency of treatment : 5 consecutive days per week for 6 weeks
Post obs. period : 2 weeks
Doses : 0 (corn oil control), 316, 562, 1,000, 1,780, 3,160 mg/kg/day

Control group : yes, concurrent vehicle
NOAEL : = 178 - 3160 mg/kg
LOAEL : = 316 - 3160 mg/kg
Method : other
Year : 1978
GLP : no data
Test substance : other TS

Method : Subchronic toxicity for National Cancer Institute Bioassay maximum tolerated dosages selection.

Result : ENDPOINTS EXAMINED: Mortality and weight changes.

LOAEL:
- Weight decrease: male mice = >3160 mg/kg/day
- Weight decrease: female mice = 316 mg/kg/day
- Mortality: male mice = 1000 mg/kg/day
- Mortality: female mice = 1000 mg/kg/day

NOAEL:
- Weight decrease: male mice = >3160 mg/kg/day
- Weight decrease: female mice = 178 mg/kg/day
- Mortality: male mice = 562 mg/kg/day
- Mortality: female mice = 562 mg/kg/day

ADDITIONAL REMARKS: No mice treated with </= 562 mg/kg/d died. In mice, the only group exhibiting mean body weight depression was the females receiving 316 mg/kg/d; body weight increased for all other groups receiving </= 1,000 mg/kg/d relative to the controls. The high dosages of 3-sulfolene selected for use in the chronic bioassay was 450 mg/kg/day for mice of both sexes.

Source : National Cancer Institute Carcinogenesis Technical Report Series No. 102, 1978.

Test condition : Test performed as a range-finder to determine maximum tolerated dose for a long-term carcinogenicity assay.

ANIMAL MAINTENANCE
- Mice were housed by sex in groups of 10 in solid-bottom polypropylene cages equipped with filter tops in temperature- and humidity-controlled rooms.
- Temperature range was 20 - 24 deg C and the relative humidity was maintained between 45 and 55 percent.
- The air conditioning system in the laboratory provided filtered air at a rate of 12 to 15 complete changes of room air per hour.
- Fluorescent lighting was provided on a 12-hour-daily cycle.

NUMBER OF ANIMALS DOSED: Six groups, each consisting of five males and five females.

GASTRIC INTUBATION
- 3-Sulfolene mixed with corn oil was introduced by gavage to five of the six mouse groups at dosages of 316, 562, 1000, 1780, and 3160 mg/kg/day.
- The sixth group served as a control, receiving only the corn oil by gavage.
-Intubation was performed for five consecutive days per week for 6 weeks on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose.
- All animals of one sex within a treated group received the same dose.

OBSERVATION PERIOD: Two weeks after termination of dosing to detect any delayed toxicity.

Test substance : 3-Sulfolene purchased from Phillips Petroleum Company. Chemical analysis performed by Hazleton Laboratories America, Inc., Vienna, Virginia. The purity of the test chemical was indicated to be approximately 92 percent.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.11.2003 (24)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma, L5178Y TK+/-, subline 3.7.2C
Concentration : 1000, 670, 449, 301, 202, 135, 90, and 61 ug/ml

Cycotoxic conc. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1982
GLP : no data
Test substance : other TS

Method : Comparable to OECD 476.

Result : SUMMARY OF MOUSE LYMPHOMA DATA FOR SULFOLENE

Results presented as Treatment & Dose Level / S-9 / % Total Survival / Mutation Frequency (x 10[-5]) / Fold Increase.

Media / - / 100.0 / 8.7 / 1.0
DMSO / - / 107.9 / 9.0 / -
EMS (620 ug/ml) / - / 25.3 / 62.0 / 7.1
1000 ug/ml / - / 112.6 / 9.7 / 1.1
670 ug/ml / - / 116.4 / 8.4 / 1.0
449 ug/ml / - / 142.5 / 6.6 / 0.8
301 ug/ml / - / 92.9 / 6.9 / 0.8
202 ug/ml / - / 115.6 / 6.8 / 0.8
135 ug/ml / - / 130.4 / 6.8 / 0.8
90 ug/ml / - / 79.9 / 10.6 / 1.0
61 ug/ml / - / 102.8 / 11.3 / 1.3
Media / + / 100.0 / 8.7 / 1.0
DMSO / + / 103.4 / 8.2 / -
MCA (3 ug/ml) / + / 76.0 / 19.2 / 2.2
1000 ug/ml / + / 57.4 / 13.1 / 1.5
670 ug/ml / + / 80.6 / 7.5 / 0.9
449 ug/ml / + / 68.6 / 10.0 / 1.1
301 ug/ml / + / 92.3 / 6.8 / 0.8
202 ug/ml / + / 90.4 / 12.4 / 1.4
135 ug/ml / + / 77.2 / 12.3 / 1.4
90 ug/ml / + / 73.8 / 13.6 / 1.6
61 ug/ml / + / 60.5 / 10.0 / 1.1

DMSO = Dimethylsulfoxide
EMS = Etylmethanesulfonate
MCA = 3-Methylcholanthrene

MUTAGENICITY EVALUATION
Exposure to 8 graded doses of Sulfolene, in the presence of and in the

absence of metabolic activation did not increase the induction of forward mutations in L5178Y Mouse Lymphoma cells at the T/K locus.

Sulfolene is considered not to be mutagenic in this test system. (author)

Source : Phillips Petroleum Company Mouse Lymphoma Forward Mutation Assay - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : TEST DESIGN
Six million preclensed TK +/- cells in six ml of F[10P] were added to each of 22 sterile, screw cap, 50 ml centrifuge tubes. An additional four ml of F[10P] were added to 11 of the tubes, and 4 ml of the S-9 mix were added to the remaining 11 tubes. Immediately thereafter, 0.1 ml of the 100X concentrations of the test chemical dilutions and the positive controls, and 0.1 ml of the solvent were added to the appropriate tubes. Each tube was mixed, gassed with a mixture of CO2 and air, and incubated at 37 +/- 0.5 deg C on a revolving roller drum for four hours. Following this, incubation tubes were centrifuged and treatment solutions decanted. Cells were washed twice with F[10P] and resuspended in 20 ml F[10P] after the second wash. The tube cultures were then gassed and reincubated as described above for a two day expression time. Growth of the cells were monitored at one and two days post-exposure and the cultures readjusted to 3.0 x10[5] cells/ml as necessary.

At the end of the expression period, a sample from each of the cultures was centrifuged, and the cells resuspended at 500,000 viable cells/ml in F[10P]. The concentrated cells were serially diluted and appropriate dilutions plated in triplicate in cloning medium with and without TFT. Approximately 500,000 viable cells (as determined by the exclusion of trypan blue) were plated on each of three selective medium plates containing 2 g/ml TFT, and 100 cells cloned on each of three non-selective plates for each test and control tube. The plates were incubated for 12 +/- 2 days. The mutant colonies (TK-/-) were counted on the selective TFT-containing plates and the survivors (TK +/- and TK-/-) were counted on the non-selective medium plates.

ACTIVATION: By an Aroclor-induced rat liver microsomal fraction

POSITIVE AND NEGATIVE CONTROLS
- Without Activation
Negative: Medium, Dimethylsulfoxide
Positive: Ethylmethanesulfonate
- With Activation
Negative: Medium, Dimethylsulfoxide
Positive: 3-Methylcholanthrene

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity, assumed 100% (Hazleton); Solubility was 100 mg/ml in cell culture medium.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
20.11.2003 (11)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains: 1535, 1537, 1538, TA98, and TA100
Concentration : 10,000; 3,333.3; 1,111.1; 370.4; and 123.5 ug/plate
Cycotoxic conc. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1982

GLP : no data
Test substance : other TS

Method : Comparable to OECD 471.

Result : Number of Revertants/Plate Following Exposure to Graded Doses of Sulfolene With and Without Metabolic Activation (Number of his+ revertants per plate, three replicate assay plates):

With Metabolic Activation:

- Negative Controls
(Strain / Organism / DMSO / DGDH2O)
1535 / 34, 14, 18 / 26, 22, 19 / 25, 30, 18
1537 / 5, 9, 6 / 7, 8, 8 / 9, 6, 5
1538 / 7, 16, 22 / 6, 13, 13 / 17, 18, 11
TA98 / 19, 14, 11 / 15, 17, 14 / 17, 19, 15
TA100 / 134, 100, 118 / 85, 107, 101 / 99, 124, 100

- Positive Controls
(Strain / MMNG [5 ug/plate] / 2-NF [50 ug/plate] / 9-AA [75 ug/plate])
1535 / 2060, 1855, 2063 / - / -
1537 / - / - / 623, 576, 708
1538 / - / 1340, 1476, 1300 / -
TA98 / - / 1641, 1708, 1630 / -
TA100 / 1871, 1997, 2067 / - / -

- 10,000 µg/plate
TA1535 - 28, 33, 31
TA 1537 - 9, 7, 8
TA1538 - 18, 18, 13
TA98 - 20, 18, 22
TA100 - 85, 125, 120

- 3,333.3 µg/plate
TA1535 - 24, 27, 35
TA 1537 - 9, 8, 6
TA1538 - 11, 24, 13
TA98 - 15, 33, 20
TA100 - 104, 96, 106

- 1,111.1 µg/plate
TA1535 - 24, 44, 23
TA 1537 - 11, 6, 9
TA1538 - 15, 8, 15
TA98 - 27, 16, 18
TA100 - 131, 104, 96

- 370.4 µg/plate
TA1535 - 31, 27, 30
TA 1537 - 11, 7, 8
TA1538 - 15, 10, 16
TA98 - 31, 20, 23
TA100 - 136, 132, 139

- 123.5 µg/plate
TA1535 - 24, 39, 22
TA 1537 - 5, 9, 7
TA1538 - 22, 8, 24
TA98 - 36, 18, 30
TA100 - 114, 118, 107

Without Metabolic Activation:

- Negative Controls
(Strain / Organism / Organism + S-9 / DMSO / DGDH2O
1535 / 34, 14, 18 / 17, 16, 14 / 9, 18, 9 / 9, 9, 8
1537 / 5, 9, 6 / 11, 5, 13 / 4, 7, 12 / 8, 6, 13
1538 / 7, 16, 22 / 26, 17, 33 / 24, 28, 30 / 12, 16, 19
TA98 / 19, 14, 11 / 30, 27, 29 / 29, 28, 24 / 26, 31, 30
TA100 / 134, 100, 118 / 125, 107, 115 / 126, 124, 95 / 107, 115, 145

- Positive Controls (ug/plate)
Strain 2-AA (5)
1535 403, 419, 367
1537 239, 220, 216
1538 1592, 1687, 1638
TA98 1715, 1860, 1741
TA100 1995, 1820, 1837

- 10,000 µg/plate
TA1535 - 14, 15, 15
TA 1537 - 8, 11, 7
TA1538 - 22, 18, 24
TA98 - 29, 28, 28
TA100 - 125, 105, 107

- 3,333.3 µg/plate
TA1535 - 17, 18, 12
TA 1537 - 6 , 11, 6
TA1538 - 31, 25, 38
TA98 - 24, 22, 27
TA100 - 120, 118, 108

- 1,111.1 µg/plate
TA1535 - 10, 18, 17
TA 1537 - 11, 7 11
TA1538 - 26, 24, 30
TA98 - 36, 35, 26
TA100 - 96, 102, 90

- 370.4 µg/plate
TA1535 - 10, 14, 20
TA 1537 - 6, 13, 9
TA1538 - 27, 24, 28
TA98 - 34, 24, 37
TA100 - 96, 115, 99

- 123.5 µg/plate
TA1535 - 12, 13, 20
TA 1537 - 16, 10, 9
TA1538 - 35, 27, 38
TA98 - 33, 28, 29
TA100 - 90, 103, 104

GENOTOXIC EFFECTS:
Negative with and without metabolic activation.

Source : Phillips Petroleum Company Salmonella typhimurium Mammalian Microsome Plate Incorporation Assay - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia

Test condition : TEST DESIGN
- 5 concentrations tested in triplicate.

- Added to 2ml of complete top agar:
--- 0.1 ml test or control substance
--- 0.1 ml overnight broth culture of each tester strain
--- 0.5 ml S9 mix (for activated portion)
- Mixed and plated on VBE minimal agar plates.
- Allowed to harden for 1 hour.
- 2 days incubation at 37±0.5 °C.
- Counted using electronic colony counter, density of background growth noted.

NUMBER OF REPLICATES: three plates per dose

FREQUENCY OF DOSING: One dose evaluated after 2 days

POSITIVE AND NEGATIVE CONTROLS
- Without Metabolic Activation
Negative: Organism, DMSO, DGDH2O
Positive: MNNG (5), 2-NF (50), 9-AA (75)
- With Metabolic Activation
Negative: Organism, Organism + S-9, DMSO, DGDH2O
Positive: 2-AA (5)

SOLVENT: dimethylsulfoxide

METABOLIC ACTIVIATION: Aroclor-induced rat liver microsomal fraction.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity, assumed 100% (Hazleton); Solubility: A homogeneous suspension containing approximately 100 mg/ml was achieved in dimethylsulfoxide.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
20.11.2003 (13)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary Cells, CCL61
Concentration : 1000, 334, 100, 34, 10 ug/ml
Cycotoxic conc. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : other TS

Method : Comparable to OECD 479.

Result : SUMMARY OF SISTER CHROMATID EXCHANGE DATA

Without Activation -- Cells (50 cells analyzed per treatment/dose).
- Results presented as Treatment & Dose Level / Total SCE's / Number of SCE's per Cell / P Value / Fold Increase in SCE's per Cell:

Media / 310 / 6.20 / - / -
H2O / 369 / 7.38 / - / -
EMS (400 ug/ml) / 953 / 19.06 / 0.0000(S) / 2.6
Sulfolene
1000 ug/ml / 296 / 5.92 / NS / 0.8
334 ug/ml / 284 / 5.68 / NS / 0.8
100 ug/ml / 329 / 6.58 / 0.06(NS) / 0.9
34 ug/ml / 374 / 7.48 / 0.43(NS) / 1.0
10 ug/ml / 328 / 6.56 / 0.06(NS) / 0.9

Without Activation -- Chromosomes
- Results presented as Treatment & Dose Level / # Analyzed / Number of SCE's per Chromosome / P Value / Fold Increase in SCE's per Chromosome:

Media / 987 / 0.32 / - / -
H2O / 997 / 0.37 / - / -
EMS (400 ug/ml) / 992 / 0.96 / 0.0000(S) / 2.6
Sulfolene
1000 ug/ml / 988 / 0.30 / NS / 0.8
334 ug/ml / 990 / 0.29 / NS / 0.8
100 ug/ml / 987 / 0.33 / 0.07(NS) / 0.9
34 ug/ml / 997 / 0.38 / 0.44(NS) / 1.0
10 ug/ml / 995 / 0.33 / 0.08(NS) / 0.9
With Activation -- Cells (50 cells analyzed per treatment/dose).
- Results presented as Treatment & Dose Level / Total SCE's / Number of SCE's per Cell / P Value / Fold Increase in SCE's per Cell:

Media / 419 / 8.38 / - / -
H2O / 410 / 8.20 / - / -
CP (1.4 ug/ml) / 906 / 18.12 / 0.0000(S) / 2.2
Sulfolene
1000 ug/ml / 323 / 6.46 / NS / 0.8
334 ug/ml / 307 / 6.14 / NS / 0.7
100 ug/ml / 390 / 7.80 / 0.26(NS) / 1.0
34 ug/ml / 363 / 7.26 / 0.04(NS) / 0.9
10 ug/ml / 397 / 7.94 / 0.32(NS) / 1.0

With Activation -- Chromosomes
- Results presented as Treatment & Dose Level / # Analyzed / Number of SCE's per Chromosome / P Value / Fold Increase in SCE's per Chromosome:

Media / 1011 / 0.42 / - / -
H2O / 985 / 0.42 / - / -
CP (1.4 ug/ml) / 1001 / 0.90 / 0.0000(S) / 2.1
Sulfolene
1000 ug/ml / 979 / 0.33 / NS / 0.8
334 ug/ml / 987 / 0.31 / NS / 0.7
100 ug/ml / 985 / 0.40 / 0.26(NS) / 1.0
34 ug/ml / 980 / 0.37 / 0.04(NS) / 0.9
10 ug/ml / 996 / 0.40 / 0.25(NS) / 1.0

EMS = Ethylmethanesulfonate
CP = Cyclophosphamide
NS = Not significant
S = Significant

MUTAGENICITY EVALUATION:
Following exposure to five graded doses of Sulfolene, no statistically significant increase in the number of SCEs per chromosome was seen at any dose level in the presence or in the absence of metabolic activation.

Sulfolene is considered not to be mutagenic in this test system. (author)

Source : Phillips Petroleum Company In vitro Sister Chromatid Exchange Chinese Hamster Ovary Cells - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia.

Test condition : TEST DESIGN
- Nonactivation:

Cells treated in an exponential stage of growth by setting up cultures with 5x105 cells per 25 cm2 flask, 24 hours prior to treatment. Cells exposed to chemical for two hours, washed twice and 5-bromodeoxyuridine (BrdU) was added to each culture. All cultures were wrapped in aluminum foil to exclude light. Cells were sampled 24 hours after addition of BrdU to ensure completion of two full cell cycles. Duplicate cultures were set up for dose level and all controls.

- Activation:
Twenty-four hours after the initiation of cultures as described above, cells were treated with the chemical in the presence of a S-9 rat liver activation system for two hours, and washed twice in saline. From this point on, cells were sampled and treated as described for the nonactivation system.

- Colecemid Administration:
Two hours prior to fixation, colecemid (0.2 g/ml) was added to each tube.

NUMBER OF REPLICATES: 2

FREQUENCY OF DOSING: exposed for two hours

POSITIVE AND NEGATIVE CONTROLS
- Without Activation
Positive: Ethylmethanesulfonate
Negative: Media Control, H2O
- With Activation
Positive: Cyclophosphamine
Negative: Media Control, H2O

EVALUATION: Fifty cells in the metaphase stage of mitosis scored at each dose level for the number of sister chromatid exchanges (SCEs). Results presented as number of SCEs per cell, and SCEs per chromosome.

Test substance : Sulfolene (2,5-dihydrothiophene 1,1-dioxide) -- no data on purity, assumed 100% (Hazleton); Solubility: Repeated vortexing was required to maintain a 200 mg/ml solution in glass distilled, deionized H2O.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.11.2003 (10)

Type : Chromosomal aberration test
System of testing : Chinese hamster ovary cells
Concentration : 0, 368, 1110, and 3680 ug/ml
Cycotoxic conc. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1990
GLP : no data
Test substance : other TS

Method : Comparable to OECD Guideline 473 - "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"

Result : Results are presented as Dose (ug/ml) / Number of Cells / Total % Cells with Aberrations

Without Activation - Test Material
0 / 200 / 3
368 / 200 / 2
1110 / 200 / 0

3680 / 200 / 1

Without Activation - Positive Control
1 / 200 / 12
5 / 50 / 20

With Activation - Test Material
0 / 200 / 2
368 / 200 / 0
1110 / 200 / 0
3680 / 200 / 1

With Activation - Positive Control
50 / 50 / 36

Results for Sulfolene were negative both with and without activation.

Source : Loveday et al., 1990.

Test condition : Cell Culture and Medium:
- CHO cells obtained from Litton Bionetics at their fifth passage level after cloning. Cells were designated CHO-LB. - Vials were stored at -80 deg C.
- Cells were not used beyond the fifteenth passage after cloning.
- Cells were tested regularly for mycoplasma contamination using 4'6-diamidino-2-phenylindole (DAPI) fluorescence and were found to be free of mycoplasma for all experiments.
- Stocks of CHO cells were maintained at 37 deg C in McCoy's 5A (modified) medium buffered with 20 mM HEPES and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 ug/ml streptomycin.
- Test cultures were set up in 75 cm2 flasks 24 hr before treatment at a uniform cell density to ensure treatment of exponentially growing cultures.

Metabolic Activation
- The rat liver microsomal fraction (S9) was prepared from Aroclor 1254-induced male Sprague-Dawley rats and was combined with cofactors and culture medium to form the metabolic activation system.

Test Chemicals:
- All test chemicals were supplied as coded aliquots by the NTP chemical repository. 3-Sulfolene was supplied by Phillips Petroleum, Chemical Division.

Controls:
- Medium and solvent controls were used with each assay. Solvent controls consisted of culture medium with or without S9 and contained the same concentration of solvent as the test cultures (0.5 or 1%).
- Positive Controls: Mitomycin C (MMC) was used in the experiments without metabolic activation, and cyclophosphamide (CP) was used in the experiments with activation.
- A single CP dose of 50 ug/ml was used in the test with S9 and an MMC dose of 5 ug/ml was used in the test without S9, these doses induced aberrations in approximately 50% of the cells.

Test Description:
- Approximately 24 hr before chemical treatment, cultures were initiated at a density of 1.75E+6/flask.
- In the trials without S9, the cultures were treated with the test chemical in medium for 8 hr, washed to remove the test chemical, and treated with colcemid (10E-6 M) for 2 to 2.5 hr before cell harvest.
- In the experiments with activation, cultures were exposed to the test chemical in serum-free medium with S9 and cofactors for 2 hr, washed to

remove the test chemical and S9, and incubated at 37 deg C with fresh medium for 8 hr. Colcemid was then added, and the cells were harvested 2 hr later.

Staining and Scoring of Slides:
- Slides were stained in 5% Giemsa for 5 min.
- 200 cells per dose were scored.
- Cells were analyzed for the following categories of chromosomal aberrations:
--- "simple" - defined as a chromatid gap, break, fragment, and deletion or chromosome gap, break, or double minutes;
--- "complex" - defined as interstitial deletions, triradials, quadriradials, rings, and dicentric chromosomes; and
---"other" - defined as pulverized chromosomes or cells with greater than 10 aberrations.
- Chromatid and chromosome gaps were recorded but were not used in the analysis.
- The frequency of polyploid or endoreduplicated cells was noted only when it seemed excessive; however, these categories were not included in the totals or in the statistical analyses.

Statistical Analysis:
- All categories of aberrations (simple, complex, and other) were combined for the statistical analysis, which was based on the percent of total cells with aberrations. The percent of aberrant cells was used for the analysis, rather than the average number of aberrations per cell.
- A binomial sampling assumption as described by Margolin et al. (1983) was used to examine absolute increased in AB's over solvent control levels at each dose. The P values were adjusted by Dunnett's method to take into account the multiple dose comparisons.
- A positive response was defined as one for which the adjusted P value was <0.05.

Test substance : 3-Sulfolene (CAS Number 77-79-2) provided by Phillips Petroleum, Chemical Div. Purity not given.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
23.12.2003 (18) (19)

5.6 GENETIC TOXICITY 'IN VITRO'

5.7 CARCINOGENITY

Species	:	rat
Sex	:	male/female
Strain	:	Osborne-Mendel
Route of admin.	:	gavage
Exposure period	:	60 to 78 weeks
Frequency of treatment	:	Five consecutive days per week
Post. obs. period	:	33 weeks
Doses	:	Males: 0, 197, and 372 mg/kg/day (time-weighted average) Females: 0, 120, and 240 mg/kg/day (time-weighted average)
Result	:	negative
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	1978

GLP : no
Test substance : other TS

Method : Route of Administration: gastric intubation

Duration of Test: 91 - 111 weeks

Doses/Concentration levels in rats:
- Each treatment group had 50 males, 50 females
- 3 sulfolene mixed in corn oil
- Males: 0 (corn oil control), 197, and 372 mg/kg/day (time-weighted average)
- Females: 0 (corn oil control), 120, and 240 mg/kg/day (time-weighted average)

Sex: male and female

Exposure Period: 60 - 78 weeks

Frequency of Treatment: five consecutive days per week

Control Group and Treatment:
- Corn oil by gavage and untreated control
- 20 males, 20 females in each control

Post exposure observation period: 33 weeks (rats)

Statistical Methods: Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extension of Cox's methods when testing a dose-related trend. The Cochran-Armitage test for linear trend in proportions with continuity correction (Armitage, 1971) and the Fisher exact test (Cox, 1970) were used to analyze potential relationships between dose and tumor formation. Life-table methods were used to analyze the incidence of tumors. Curves of the proportion surviving without an observed tumor were computed as in Saffiotti et al. (1972).

Endpoints Examined: Mortality, weight changes, pathology/tumor incidence

Remark : Administration of 3 sulfolene via gastric intubation to Osborne-Mendel rats resulted in early mortality, which was associated with the occurrence of a variety of non-neoplastic lesions. Neoplasms that were observed occurred in incidences that were within or below the range of spontaneous incidence observed in Osborne-Mendel rats.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 3 sulfolene to Osborne-Mendel rats.

Result : LOAEL (LOEL):
- Weight decrease: male rats = 372 mg/kg/day; weight decrease not observed in female rats
- Mortality: male rats = 197 mg/kg/day; female rats = 240 mg/kg/day

NOAEL (NOEL)
- Weight decrease: male rats = 197 mg/kg/day; female rats = 240 mg/kg/day
- Mortality: male rats = <197 mg/kg/day; female rats = 120 mg/kg/day

Statistical results:
- For male rats, the Tarone test indicated a significant ($P < 0.001$) positive

association between dosage and mortality when dosed groups were compared to the vehicle controls. Due to the accelerated mortality in the high dose group, the departure from linear trend was also significant (P < 0.001).
- For female rats, the Tarone test showed a significant (P = 0.002) positive association between dosage and mortality when dosed groups were compared to the vehicle controls. The Cochran-Armitage test indicated a significant (P = 0.029) negative association between dose and the incidence of pituitary chromophobe adenomas; the Fisher exact test was not significant for this type of tumor.

Remarks for results: Evidence of toxicity that led to accelerated mortality was morphologically reflected primarily in the circulatory, urinary, biliary, and reproductive systems.

Source : National Cancer Institute Carcinogenesis Technical Report Series No. 102, 1978.

Test substance : 3-Sulfolene (NCI number C04557), CAS Number 77-79-2, 92% purity.

Reliability : (2) valid with restrictions
19.12.2003 (1) (3) (4) (16) (22) (23) (24)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 60-78 weeks
Frequency of treatment : five consecutive days per week
Post. obs. period : 13 weeks
Doses : Males: 0 (corn oil control), 311, and 622 mg/kg/day (time-weighted average)
Females: 0 (corn oil control), 384, and 768 mg/kg/day (time-weighted average)

Result : negative
Control group : yes, concurrent vehicle
Method : other
Year : 1978
GLP : no
Test substance : other TS

Method : Route of Administration: gastric intubation

Duration of Test: 91 - 111 weeks

Doses/Concentration levels in mice:
- Each treatment group had 50 males, 50 females
- 3 sulfolene mixed in corn oil
- Males: 0 (corn oil control), 311, and 622 mg/kg/day (time-weighted average)
- Females: 0 (corn oil control), 384, and 768 mg/kg/day (time-weighted average)

Sex: male and female

Exposure Period: 60 - 78 weeks

Frequency of Treatment: five consecutive days per week

Control Group and Treatment:

- Corn oil by gavage and untreated control
- 20 males, 20 females in each control

Post exposure observation period: 13 weeks (mice)

Statistical Methods: Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extension of Cox's methods when testing a dose-related trend. The Cochran-Armitage test for linear trend in proportions with continuity correction (Armitage, 1971) and the Fisher exact test (Cox, 1970) were used to analyze potential relationships between dose and tumor formation. Life-table methods were used to analyze the incidence of tumors. Curves of the proportion surviving without an observed tumor were computed as in Saffiotti et al. (1972).

Endpoints Examined: Mortality, weight changes, pathology/tumor incidence

Remark : The administration of the high dose of 3 sulfolene increased mortality in mice of both sexes, thus the potential carcinogenic effect could not be evaluated in these groups. Survival of animals receiving low doses was believed to be sufficient to conclude that there was no tumorigenic effect at that concentration.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 3 sulfolene to B6C3F1 mice.

Result : LOAEL (LOEL):
- Weight decrease: not observed in male and female mice
- Mortality: male mice = 622 mg/kg/d; female mice = 240 mg/kg/d

NOAEL (NOEL):
- Weight decrease: male mice = 622 mg/kg/day; female mice = 768 mg/kg/d
- Mortality: male mice = 311 mg/kg/d; female mice = 120 mg/kg/d

Statistical results:
- For male mice, the Tarone test indicated a significant (P < 0.001) positive association between dosage and mortality when comparing the dosed group to the vehicle control. Due to the accelerated mortality in the high dose group, the departure from linear trend was also significant (P <0.001). The Cochran-Armitage test indicated a significant (P = 0.040) positive association between dose and incidence of hepatocellular carcinomas; the Fisher exact test was not significant.
- For female mice, the Tarone test showed a significant (P < 0.001) positive association between dosage and mortality when comparing the dosed groups to the vehicle control. The accelerated mortality in the high dose group resulted in significant (P < 0.001) departure from linear trend.

Remarks for results: Evidence of toxicity that led to accelerated mortality was morphologically reflected primarily in the circulatory, urinary, biliary, and reproductive systems.

Source : National Cancer Institute Carcinogenesis Technical Report Series No. 102, 1978.

Reliability : (2) valid with restrictions
19.12.2003 (1) (3) (4) (16) (22) (23) (24)

5.8 TOXICITY TO REPRODUCTION

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

201-15167B2

Appendix II

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Sulfolane
Robust Summaries

Existing Chemical	:	Sulfolane
CAS No.	:	126-33-0
EINECS Name	:	
EC No.	:	
Molecular Formula	:	C4H8O2S

Producer related part

Company	:	
Creation date	:	

Substance related part

Company	:	
Creation date	:	
Status	:	
Memo	:	
Printing date	:	
Revision date	:	
Date of last update	:	
Number of pages	:	
Chapter (profile)	:	
Reliability (profile)	:	
Flags (profile)	:	

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1.5×10^{6} OH/cm^3
Rate constant	:	= 1.32785×10^{-11} cm^3/(molecule*sec)
Degradation	:	= 50 % after 9.7 hour(s)
Deg. Product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	no data

Method : Calculated by using AOPWIN (ver.1.90), based on the Atkinson model recommended in the OECD Guidance.

Conclusion : The substance in air is indirectly photodegraded with half-life of 9.7 hours.

Reliability : (2) valid with restrictions
The value is estimated with the method recommended in the OECD Guidance.

Flag : Critical study for SIDS endpoint

18.07.2001 (b)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	1999
GLP	:	No

Result : Nominal: ca.1000 mg/L
Degradation: No hydrolysis at pH 4,7 and 9 at 50±1°C for 5 days.

Test substance : other TS; Produced by Nacalai Tesque Inc., Lot No. M6E9460, Purity: 99.6%

Conclusion : The substance is very stable at pH4, 7 and 9 at 50±1°C for 5 days.

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

Flag : Critical study for SIDS endpoint

18.07.2001 (a)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model Mackay level III
Media :
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method :
Year : 2002
Method : The parameters used are shown in Appendix Ⅰ.
Result : Estimated Distribution under three emission scenarios

Compartment	Release 100% to air	100% to water	100% to soil	equally to each compartment
Air	0.3%	0.0%	0.0%	0.1%
Water	50.7%	99.6%	45.2%	59.0%
Soil	48.8%	0.0%	54.6%	40.7%
Sediment	0.2%	0.4%	0.2%	0.2%

Attached doc. : Appendix: Parameters used in calculation of distribution by Mackay level Ⅲ fugacity model.

Conclusion : The majority of the substance would distribute into water if released to aquatic compartment, and water and soil if released into air or soil and if released equally to each compartment.

Reliability : (1) valid without restriction
The model employed is developed by the Japanese government.
Flag : Critical study for SIDS endpoint
18.07.2001 (c)

Appendix 1 : Parameters used in calculation of distribution by Mackay level Ⅲ fugacity model.

Physico-chemical parameter

molecular weight		120.17	Measured
melting point [°C]		27	Measured
vapor pressure [Pa]		1.30E+00	Measured
water solubility [g/m^3]		100000	Measured
log Kow		-0.77	Measured
half life [h] (Note 1)	in air	9.7	Estimated
	in water	240000	Estimated
	in soil	240000	Estimated
	in sediment	720000	Estimated

Temp. [°C]	25

Environmetal parameter

		volume [m^3]	depth [m]	area [m^2]	organic carbon [－]	lipid content [－]	density [kg/m^3]	residence time [h]
bulk air	air	1.0E+13					1.2	100
	particles	2.0E+03						
	total	1.0E+13	1000	1E+10				
bulk water	water	2.0E+10					1000	1000
	particles	1.0E+06			0.04		1500	
	fish	2.0E+05				0.05	1000	
	total	2.0E+10	10	2E+09				
bulk soil	air	3.2E+08					1.2	
	water	4.8E+08					1000	
	solid	8.0E+08			0.04		2400	
	total	1.6E+09	0.2	8E+09				
bulk sediment	water	8.0E+07					1000	
	solid	2.0E+07			0.06		2400	50000
	total	1.0E+08	0.05	2E+09				

Intermedia Transport Parameters［m/h］

air side air-water MTC	5	soil air boundary layer MTC	5
water side air water MTC	0.05	sediment-water MTC	1E-04
rain rate	1E-04	sediment deposition	5E-07
aerosol deposition	6E-10	sediment resuspension	2E-07
soil air phase diffusion MTC	0.02	soil water runoff	5E-05
soil water phase diffusion MTC	1E-05	soil solid runoff	1E-08

(Note 1) The half life in air is estimated by using AOPWIN (ver.1.90).
The default values applied for other half lives, as recommended by Chemicals Evaluation and Research Institute, Japan.

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge
Concentration : 100mg/L related to test substance
Contact time : 14days
Degradation : =10%after 14 days
Result : Under test conditions no biodegradation observed
Control substance : Aniline
Kinetic :

Deg. Product : Not measured
Method : OECD Guide-line 301 C"Ready Biodegradability: Modified MITI Test(Ⅰ)
Year : 1975
GLP : No
Result : 10% after 14 days(based on BOD)

Test substance : Other TS

Conclusion : The substance is not readily biodegradable.

Reliability : (1)valid without restriction
The data is approved by the Japanese government.
Flag : Critical study for SIDS endpoint
18.07.2001 d

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : *Cyprinus carpio* (Fish, fresh water)
Exposure period : 6 weeks at 25°C
Concentration : Yes
Elimination : No
Method : OECD Guide-line 305 E"Bioaccumulation: Flow-through Fish Test"
Year : 1977
GLP : No
Test substance :
Remark :
Result : Bioconcentration Factor:

Exposure conc.	2 week	3 week	4 week	6week
2.5 mg/L	0.7, 0.6	0.6, 0.5	0.8, 0.5	0.4, 0.4
0.25 mg/L	<13, <13	<13, <13	<13, <13	<13, <13

Test condition : Test concentrations: 2.5 mg/L and 0.25 mg/L
The stock solution (10,000 mg/L) for exposure was prepared by disolving the test substance in water. The exposure was conducted under flow-through conditions.

Test substance	:	Other TS
Conclusion	:	The BCF of the chemical is 0.4-0.8(2.5mg/L)and <13(0.25mg/L).
Reliability	:	(1)valid without restriction The data is approved by the Japanese government.
Flag 18.07.2001	:	Critical study for SIDS end point e

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : semistatic
Species : *Oryzias latipes* (Fish, fresh water)
Exposure period : 96 hour(s)
Analytical monitoring : yes
LC0 : >= 100 mg/L
LC50 : > 100 mg/L
LC100 : > 100 mg/L
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1999
GLP : yes
Test substance : other TS; Produced by Wako Pure Chemical Industries, Ltd. Lot No. PAH5988, Purity: 95.8%

Method :
-Test organisms:
a) Size (scaled body length and body weight): 1.8-2.0 cm, 0.083-0.12 g (n=10)
b) Age: not described
c) Pretreatment: Acclimated for more than 12 days at the same conditions of the test
d) Supplier/Source: Nakajima Aquaculture (Kumamoto Prefecture, Japan)
-Test conditions:
a) Dilution water source: Dechlorinated tap water
b) Dilution water chemistry: hardness=52.0 mg/L as $CaCO_3$, pH=7.5
c) Exposure vessel type: 3 L volume glass aquarium (16 cm in diameter x 17 cm depth) with a lid
d) Nominal concentrations: 0, 100 mg/L (Limit test)
e) Vehicle/solvent and concentrations: Not used
f) Stock solutions preparations and stability: Appropriate amount of test substance was dissolved with dilution water and 10,000 mg/L stock solution was prepared. Test solution was prepared by mixing appropriate amount of the stock solution and dilution water.
g) Number of replicates: 2
h) Individuals per replicates: 5
i) Loading: Approximately 4.5 L of water was used for 1 g of fish
j) Dosing rate, flow-through rate:
k) Renewal frequency of test water: Semistatic with 48 hours interval
l) Water temperature: 24±1°C
m) Light condition: 16 hours light/8 hours dark (room light)
n) feeding: no
-Method of analytical monitoring: GC (at start and just before renewal of test water)
-Statistical method:
a) Data analysis: Binomial method
b) Method of calculating mean measured concentrations: Time-weighted mean

Result :
-Measured concentrations:

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percent of nominal) 0-hour(a)	48-hour(b)	Mean(c)
control	n.d.	n.d.	–
100	97.8 (97.8)	103 (103)	100 (100)

n.d:<10.0mg/L
(a)fresh solution, (b) expired solution
(c)The values are expressed as time-weighted means.

-Water chemistry in test: Water temperature=24.2-24.8°C, pH=7.2-7.3, DO=6.9-8.3 mg/L

-Cumulative mortality:

Nominal concentration (mg/L)	Cumulative number of dead fish (Percent mortality)			
	24-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)

-Statistical result: 96-hour LC_{50} >100 mg/L.

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

Flag : Critical study for SIDS endpoint

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4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Endpoint : Immobility
Species : *Daphnia magna* (Crustacea)
Exposure period : 48 hour(s)
Analytical monitoring : yes
NOEC : 171 mg/L
EC_{50} : 852 mg/L
Method : OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year : 1999
GLP : yes
Test substance : other TS; Produced by Wako Pure Chemical Industries, Ltd. Lot No. PAH5988, Purity: 95.8%

Method : -Test organisms:
a) Age: <24 hours after hatch
b) Pretreatment:
c) Supplier/Source: Laboratory cultures maintained at Chemical Inspection and Testing Institute,Japan
-Test conditions:
a) Dilution water source: Dechlorinated tap water
b) Dilution water chemistry: hardness=52.0 mg/L as CaCO3, pH=7.5
c) Exposure vessel type: Petri dish (8.5 cm diameter x 5.7 cm depth)
d) Nominal concentrations: 0, 95.3, 171, 309, 556, 1000 mg/L
e) Vehicle/solvent and concentrations: Not used
f) Stock solutions preparations and stability: Appropriate amount of test substance was dissolved with dilution water and 10,000 mg/L stock solution was prepared. Test solution was prepared by mixing appropriate amount of the stock solution and dilution water and divided into 4 vessels.
g) Number of replicates: 4
h) Individuals per replicates: 5
i) Volume of test solution: 200mL/vessel
j) Renewal rate of test water: no
k) Water temperature: 20±1°C
l) Light condition: 16 hours light/8 hours dark (room light intensity)
m) feeding: no
-Method of analytical monitoring: GC (at start and end of test)
-Statistical method:

a) Data analysis: Probit methods
b) Method of calculating mean measured concentrations: Time-weighted mean

Result : -Measured concentrations

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percent of nominal) 0-hour(a)	48-hour(b)	Mean(c)
control	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)
1,000	1,010(101)	936(93.6)	972(97.2)

n.d:<10.0 mg/L
(a)fresh solution, (b) expired solution
(c)The values are expressed as time-weighted means.

-Water chemistry in test: Water temperature=20.2-20.5°C, pH=7.8-7.9, DO=8.7-8.8 mg/L

-Cumulative immobilization:

Nominal concentration (mg/L)	Cumulative number of Immobilized Daphnia (Percent immobility) 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)
1,000	12 (60)	13 (65)

The values include dead Daphnia.

-Statistical result: 24 and 48-hour EC_{50}=889 and 852 mg/L

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

Flag : Critical study for SIDS endpoint

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4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : *Selenastrum capricornutum* (Algae)
Endpoint : Biomass and growth rate
Exposure period : 72 hour(s)
Analytical monitoring : yes
NOEC (biomass) : 171 mg/L
NOEC (growth rate) : 309 mg/L (24-48h)
EC_{50} (biomass) : 500 mg/L
EC_{50} (growth rate) : >1,000 mg/L
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1999

GLP : yes

Test substance : other TS; Produced by Wako Pure Chemical Industries, Ltd.
Lot No.PAH5988,Purity: 95.8%

Method : -Test organisms:
a) Supplier/Source (strain number): Laboratory cultures maintained at Chemical Inspection and Testing Institute, Japan/ATCC 22662
b) Preculture (duration, medium, etc.): 3 days under the same method of test in OECD medium
-Test conditions:
a) Test medium: OECD medium
b) Exposure vessel type: 500 mL volume glass Erlenmeyer flask
c) Nominal concentrations: 0, 95.3, 171, 309, 556, 1000 mg/L
d) Vehicle/Solvent and concentrations: not used
e) Stock solutions preparations and stability: Appropriate amount of test substance was dissolved with OECD medium and 10,000 mg/L stock solution was prepared. The stock solution was sterilized by filtration with 0.45 µm membrane filter. Test solution was prepared by mixing appropriate amount of the stock solution and OECD medium.
f) Number of replicates: 3
g) Initial cell number (initial biomass): $1x10^4$ per mL
h) Volume of test solution: 100mL/vessel
i) Water temperature range: 23±2°C
j) Light condition (intensity, duration): 4000-5000 lux, continuous
-Method of analytical monitoring: GC (at start: test solution from another vessel for analysis, and at end: Centrifuged supernatant of mixed test solution from 3 test vessels)
-Statistical method:
a) Data analysis: Comparison of areas under the growth curves and growth rate. NOEC: one-way ANOVA and Dunnett's multiple comparison. EC_{50}: method of least squares
b) Method of calculating mean measured concentrations: Time-weighted mean

Result : -Measured concentrations

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percent of nominal) 0-hour(a)	72-hour(b)	Mean(c)
control	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)
1,000	976(97.6)	916(91.6)	946(94.6)

n.d:<10.0mg/L
(a)initial, (b) final
(c)The values are expressed as time-weighted means.

-Water chemistry in test: Water temperature=22.5-24.9°C, pH=7.9-8.0 at the initiation of exposure and 8.6-9.9 at the termination of exposure

-Cell concentration at each flask of each measuring point:

Nominal Concentration (mg/L)	Cell density (x10E+4 cells/mL) No.	0-hour	24-hour	48-hour	72-hour
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
	S.D	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
	S.D	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
	S.D	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
	S.D	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
	S.D	0.0	0.5	4.4	10.7
1,000	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
	S.D	0.0	0.3	4.0	21.1

-Growth inhibition:

Nominal Concentration (mg/L)	No.	Area (X10E+4) 0-72h	Inhibition (%) 0-72h	Rate 24-48h	Inhibition (%) 24-48h	Rate 24-72h	Inhibition (%) 24-72h
Control	1	3760	-	0.0831	-	0.0720	-
	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	2670	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
1,000	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	7 1.1	0.0500	42.7	0.0517	30.0

The control group showed normal growth (more than 200-fold increase after 72hr). The growth was inhibited at 1,000 mg/L, 556 mg/L and 0.640 mg/L. The lower concentration groups showed similar growth to the control.

-Statistical result: EbC_{50}(0-72 h)= 500 mg/L (95% confidence limits: 416 – 601 mg/L) and NOEbC=171 mg/L.
ErC_{50}(24-48 h) >1,000 mg/L and NOECr(24-48h)=309mg/L. ErC_{50}(24-72 h) >1,000 mg/L and NOErC(24-72h)=556 mg/L.

Reliability : (1) valid without restriction
The data is approved by the Japanese government.
Flag : Critical study for SIDS endpoint
14.09.2001 h

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
Analytical monitoring : yes
NOEC : 25.0 mg/L
LCEC : 50.0 mg/L
EC_{50} : >100 mg/L
LC_{50} : >100 mg/L
Method : OECD Guide-line 211 "Daphnia magna reproduction test" (Draft Guideline, April 1997)
Year : 1999
GLP : yes

Test substance : other TS; Produced by Wako Pure Chemical Industries, Ltd.
Lot No.pah5988,Purity: 95.8%

Method : -Test organisms:
a) Age: <24 hours after hatch
b) Pretreatment:
c) Supplier/Source: Laboratory cultures maintained at Chemical Inspection and Testing Institute, Japan
-Test conditions:
a) Dilution water source: Dechlorinated tap water
b) Dilution water chemistry: hardness=52.0 mg/L as $CaCO_3$, pH=7.5
c) Exposure vessel type: 100 mL volume glass beaker
d) Nominal concentrations: 0, 25.0, 50.0, 100 mg/L
e) Vehicle/solvent and concentrations: Not used
f) Stock solutions preparations and stability: Appropriate amount of test substance was dissolved with dilution water and 1000 mg/L stock solution was prepared. Test solution was prepared by mixing appropriate amount of the stock solution and dilution water and divided into 10 vessels.
g) Number of replicates: 10
h) Individuals per replicates: 1
i) Volume of test solution: 500 mL/vessel
j) Renewal frequency of test water: Semistatic with 3 times per week
k) Water temperature: 20±1°C
l) Light condition: 16 hours light/8 hours dark (room light intensity)
m) feeding: Chlorella vulgaris, 0.1-0.2 mg organic carbon/individual/day
-Method of analytical monitoring: GC (just before and after renewal of test water x 3)
-Statistical method:
a) Data analysis: Kruskal-Wallis, Dunnett multiple comparisons method for dead parental Daphnia. Kruskal-Wallis for time of the first production of young. Analysis of variance in one-way classification and Scheffe's multiple comparisons for mean cumulative numbers of young production per adult.
b) Method of calculating mean measured concentrations: Time-weighted mean

Result : -Measured concentrations:

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percent of nominal)			
	0-day(a)	2-day(b)	11-day(a)	14-day(b)
control	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)
50.0	50.8(102)	49.1(98.2)	47.9(95.9)	51.2(102)
100	99.1(99.1)	101(101)	97.7(97.7)	103(103)

(continued)

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percent of nominal)		
	16-day(a)	18-day(b)	Time-weighted mean(c)
control	n.d.	n.d.	n.d
25.0	25.6(102)	24.2(97.0)	24.6(98.3)
50.0	51.8(104)	48.8(97.6)	49.9(99.7)
100	106(106)	96.7(96.7)	101(101)

n.d:<10.0mg/L
(a)fresh solution, (b) expired solution
(c)The values are expressed as time-weighted means.

-Water chemistry in test: Water temperature=20.0-20.3°C, pH=7.5-8.6, DO=8.3-8.8 mg/L, hardness=43.0-49.0 mg/L as $CaCO_3$

-Cumulative number of dead parental Daphnia:

Nominal concentration (mg/L)	Exposure time (day) 3	7	14	21
control	0 (0)	0 (0)	0 (0)	0 (0)
25.0	0 (0)	0 (0)	0 (0)	0 (0)
50.0	0 (0)	0 (0)	0 (0)	4 (40)
100	0 (0)	0 (0)	0 (0)	4 (40)

The values in parentheses express mortality (%) of Daphnia.

-Time of the first production of young: 8 days in all test groups

-Mean cumulative numbers of young production per adult:

Nominal concentration (mg/L)	Exposure time (day) 7	8	9	10	11	12	13	14
control	0	21.2	21.3	21.3	62.0	62.0	62.0	106
25.0	0	20.0	20.0	20.0	55.9	55.9	55.9	96.6
50.0	0	18.7	18.7	18.7	53.0	53.0	53.0	94.3
100	0	19.5	19.5	19.5	51.5	51.5	51.5	77.5

(continued)

Nominal concentration (mg/L)	Exposure time (day) 15	16	17	18	19	20	21
control	106	106	109	146	146	146	178
25.0	97.2	97.2	109	132	132	136	155
50.0	94.3	94.5	107	132	132	132	160
100	80.2	80.2	80.2	93.0	93.0	93.0	96.5

-Significant test of difference between the mean cumulative number of juveniles produced per adult in control and test vessels after 21 days exposure:

Nominal concentration (mg/L)	Vessel No. 1	2	3	4	5	6	7
control	184	190	185	187	169	186	183
25.0	133	136	159	196	171	180	116
50.0	-	113	186	170	176	-	172
100	106	18	-	145	-	101	115

(continued)

Nominal concentration (mg/L)	Vessel No. 8	9	10	Mean	S.D.	Significant difference

control	170	202	128	178	20.1	
25.0	109	185	168	155	30.1	
50.0	-	141	-	160	27.4	
100	-	94	-	97	42.4	**

**: Significantly different from Control at p<0.01.

-Statistical result: 14 and 21-day LC50 for dead parental Daphnia>100 mg/L, 14 and 21-day EC50 for reproduction>100 mg/L, 21-day NOEC and LOEC for reproduction=25.0 and 50.0 mg/L based on the nominal concentrations.

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

Flag : Critical study for SIDS endpoint

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4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Acute Oral Toxicity

TEST SUBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No. 126-33-0)
- Remarks: Source: Produced by Shin Nippon Rika , Lot No:0007 ,Purity 95 %
  (other constituent: Water 5 %)

METHOD

- **Method/guideline:** OECD Guideline 401
- **Test type:** Acute Oral Toxicity Test
- **GLP:** Yes
- **Year:** 1996 (published year)
- **Species:** Rat
- **Strain:** Crj: CD(SD)
- **Route of administration:** Oral(by single-dose gavage)
- **Dose/concentration levels:** 0, 892, 1204, 1626, 2195, 2963, 4000 mg/kg
- **Sex:** Male & Female
- **Control group and treatment:** Vehicle
- **Post exposure observation period:** 14 days
- **Statistical methods :** LD_{50} was calculated by Van der Waerden method

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- **Age at study initiation:** 5 week old for both sexes
- **Weight at study initiation:** 122-138g for males, 107-120g for females
- **No. of animals per sex per dose:** 5 per sex per dose group

Study Design:

- **Vehicle:** Purified water

- **Satellite groups and reasons they were added:** none
- **Clinical observations performed and frequency:** Each rat was weighed immediately prior to treatment, the day and thereafter 1, 3, 7 and 14 days after treatment. The rats were observed periodically during two-week post-treatment observation period for signs of toxicity. All rats were submitted for a gross pathological examination.

RESULTS

- **LD_{50}:**
  Male: 2006 mg/kg b.w. (1783-2256 mg/kg b.w., 95% confidence limit)
  Female: 2130 mg/kg b.w. (1844-2460 mg/kg b.w., 95% confidence limit)

REMARKS FIELD FOR RESULTS

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

- **Food consumption:** Not done
-
- **Clinical signs** (description, severity, time of onset and duration): Signs of
- toxicity were observed at the dose group more than 1204 mg/kg, including convulsion, decreased locomotor activity, ptosis, salivation, piloerection, chromodacryorrhea and perineal region soiling with urine.

  Decreased locomotor activity; more than 1204 mg/kg
  Convulsion, ptosis, salivation, piloerection, chromodacryorrhea, perineal region soiling with urine; more than 1626 mg/kg

- **Hematology :** Not done
- **Biochem:** Not done
- **Ophthalmology:** Not done

Mortality and time to death:

Mortality of male and female rats

Dose level (mg/kg	#Dead/#Treated		Time of death	
	Males	Females	Males	Females
0	0/5	0/5	-	-
892	0/5	0/5	-	-
1204	0/5	0/5	-	-
1626	0/5	0/5	-	-
2195	4/5	3/5	Day 0 4/5	Day 0 3/5
2963	5/5	5/5	Day 0 5/5	Day 0 5/5
4000	5/5	5/5	Day 0 5/5	Day 0 5/5

- **Gross pathology incidence and severity:** Dead animals showed hemorrhagic black spots in their glandular stomach mucosa at necropsy.
- **Organ weight changes:** Not done
- **Histopathology (incidence and severity):** Not done

CONCLUSIONS

LD_{50} was established at 2006 mg/kg for male and 2130 mg/kg for female, respectively.

DATA QUALITY

- **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carries out by Research Institute for Animal Science in Biochemistry and Toxicology

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals. 4, 435-436 (1996)

GENERAL REMARKS

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Repeated Dose Toxicity (a)

TEST SUBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No. 126-33-0)
- Remarks: Source: Shinn Nippon Rika, Lot No:0007, Purity 95 % (other constituent: water 5%, Kept at 4° C until use

METHOD

- **Method/guideline:** TG for 28-day repeat dose toxicity testing of Chemicals(Japan)
- **Test type:** 28-day Repeat Dose Toxicity Test
- **GLP:** Yes
- **Year:** 1996 (published year)
- **Species:** Rat
- **Strain:** Crj: CD(SD)
- **Route of administration:** Oral(gavage)
- **Dose/concentration levels:** 0, 60, 200, 700 mg/kg/day
- **Sex:** Male & Female
- **Exposure period:** Males and females, for 28 days
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** 14 days (for 0 and 700 mg/kg/day group)
- **Terminal killing:** Males and females, days 29 or 43
- **Statistical methods :** Dunnett's test
  t-test and u-test for data of recovery group

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- **Age at study initiation:** 5 week old for both sexes
- **Weight at study initiation:** 146-157g for male, 126-140g for female
- **No. of animals per sex per dose:** 6 per sex per dose for the group at 60, 200 mg/kg/day, and 12 per sex per dose for the group at 0, 700 mg/kg/day

Study Design:

- **Vehicle:** Purified water
- **Satellite groups and reasons they were added:** none
- **Clinical observations performed and frequency:** General condition was observed once a day. Body weight and food consumption were determined at day 1, 3 after treatment, and thereafter twice a week. Hematological and blood chemical analysis were carried out at the day 28 (all groups) and at the 43 (control and 700 mg/kg :recovery group). Urinary analysis were carried out at the day 23 (male), 24 or 27 (female) for all group, and at the day 34 (male) and 32 (female) for control and 700 mg/kg (recovery group).
- **Organs examined at necropsy:**
  Organ weight: brain, heart, liver, kidneys, spleen, thymus, adrenal glands, testes, epididymides, ovary
  Microscopic: control & all treated groups(male)/ kidney control & 700mg/kg groups/brain, spinal cord, pituitary, eye ball, thyroid, thymus, heart, trachea, lung, liver kidneys, spleen, adrenals, stomach, small intestines, pancreas, testes, epididymides, prostate, ovary, uterus, vagina, bladder, lymph node, sciatic nerve, bone marrow

RESULTS

- NOAEL

  60 mg/kg/day for males and 200 mg/kg/day for females

- LOAEL

  Male: 200 mg/kg/day (histopathological changes in the kidney)
  Female: 700 mg/kg/day (suppression of body weight gain & food consumption, and slight increase of GPT value in blood chem.)

REMARKS FIELD FOR RESULTS

Body weight: Decrease of body weight gain in 700 mg/kg males and females

- **Food consumption:** Decrease in 700 mg/kg males and females
- **Clinical signs** (description, severity, time of onset and duration):Transient reduction of locomotor activity at the early stage in 700mg/kg females

Mortality and time to death: The death was not observed in any group.

- **Hematology and biochemical findings:**

Male: Slight decrease in MCHC at all groups(P<0.05~P<0.01), but no dose-related, and no changes in erythrocytes, hemoglobin, and hematocrit values. Increase in leukocyte at 700mg/kg of recovery group (P<0.05).

Female: Decrease in erythrocytes and increase in MCV at 700mg/kg of recovery group (P<0.01).

Hematology examinations in male and female rats

	28days dosing group				14 days 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
leukocytes (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 (fl)	57±2	57±2	57±1	58±1	55±1	57±1**

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

- **Blood chemistry:**

Male: Increase of cholinesterase (P<0.05) and total bilirubin (P<0.01) ,and decrease of chloride (P<0.01) at 700mg/kg

Female: Increase of GPT ((P<0.01) and decrease of glucose (P<P0.05) at 700 mg/kg

Blood chemical examinations in male and female rats

	28days dosing group				14days 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/dl)	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/dl)	130±15	117±13	123±10	110±4*	139±13	125±10

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

- **Gross pathology incidence and severity:** Slight hypertrophy of kidney
- at 700 mg/kg males (2/6)
- **Organ weight changes :**

Male: Increase of relative weight of kidneys (P<0.01), brain and heart (P<0.05) at 700 mg/kg

Female: Decrease of splenic weight (P<0.05) at 700 mg/kg. Increase of absolute and relative weight of spleen

(P<0.01) at 700 mg/kg of recovery group

Organ weight in male and female rats

	28days dosing group				14days 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(g%)	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 wt.						
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 wt.						
Spleen	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)

- **Histopathology** (incidence and severity):
  Male: Increase of hyaline droplets and eosinophilic bodies in the renal tubules in 200 and 700 mg/kg. Increase of basophilic renal tubules at 700 mg/kg
  Female: No significant effect was observed.

CONCLUSIONS

The NOAELS are considered to be 60 mg/kg/day for males and 200 mg/kg for females.

DATA QUALITY

- **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carries out by Research Institute for Animal Science in Biochemistry and Toxicology (Japan)

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 4, 437-445 (1996)

GENERAL REMARKS

Repeated Dose Toxicity（b）

TEST SUBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No. 126-33-0)
- Remarks: Source: Produced by Sumitomo Seika Chemicals, Lot No.20802
  Purity: 99.3%, kept at room temperature until use

METHOD

- **Method/guideline:** OECD TG 421
- **Test type:** OECD Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1999(published year)
- **Species:** Rat
- **Strain:** Crj: CD(SD)IGS
- **Route of administration:** Oral(by gavage)
- **Dose/concentration levels:** 0, 60, 200, 700 mg/kg/day(in Water)
- **Sex:** Male & Female
- **Exposure period:** Male; for 49 days
  Female; for 41-50 days from 14 days prior to mating to the 3 day of lactation
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** none
- **Terminal killing:** Males; day 50
  Females; day 4 of lactation
- **Statistical methods :** Dunnett's)following to Bartlett or Kruskal-Wallis analysis) and Chi square test

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- **Age at study initiation:** 10 week old for both sexes
- **Weight at study initiation:** 355-379g for males, 209-228g for females
- **No. of animals per sex per dose:** 12 per sex per dose group

Study Design:

- **Vehicle:** Water
- **Satellite groups and reasons they were added:** none
- **Clinical observations performed and frequency:** General condition was observed twice a day. Body weight and food consumption for males were determined twice a week, and for females, body weight and food consumption were determined twice a week prior to mating and in principle, once a week for gestation and lactation period.
- **Organs examined at necropsy:**
  Organ weight: testes, epididymides, ovaries
  Microscopic: control & 700 mg/kg groups/ testis, epididymides, ovary

RESULTS

- NOAEL

  200 mg/kg/day in both sexes

- LOAEL

  Both sexes: 700 mg/kg/day (death, clinical toxic signs, suppression of body weight gain)

REMARKS FIELD FOR RESULTS

- **Body weight:** Suppression of body weight gain was observed in the 700 mg/kg male and during pre-mating and lactation period in the 700 mg/kg female.
- **Food consumption:** Decrease in food consumption was observed in the 700 mg/kg male, and during pre-mating and lactation period in the 700 mg/kg female.
- **Clinical signs** (description, severity, time of onset and duration):Soiled fur for both sexes and soft stool for males in the 700 mg/kg group were noted.
  Mortality and time to death: One male and one female in the 700 mg/kg group died.
- **Hematology and biochemical findings:** Not done

- **Gross pathology incidence and severity:** No significant effect was observed.
- **Organ weight changes :**
  Male: No significant effect was observed.
  Female: The relative ovary weights were increased in the 700 mg/kg group.

Ovary weights

Dose level (mg/kg)	0	60	200	700
No. of dams	12	12	12	12
Ovaries				
Relative (mg%)	32.90±4.36	33.04±4.62	34.66±3.33	40.45±5.92**

(**:P<0.01)

- **Histopathology (incidence and severity):** No significant effect was observed.

CONCLUSIONS

Toxic effects in this study are death, clinical toxic signs, suppression of body weight gain and low food consumption.

The NOAELS are considered to be 200 mg/kg/day for both sexes.

DATA QUALITY

・ **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Nihon Bioresearch Inc. Hashima Laboratory (Japan)

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 7, 471-481 (1999)

GENERAL REMARKS

5.5 GENETIC TOXICITY 'IN VITRO'

GENETIC TOXICITY IN VITRO (BACTERIAL TEST)

TEST SUBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No. 126-33-0)
- Remarks: Source: Produced by Shinn Nippon Rika, Lot No.8050074 , Purity:
- above 99.9%, Kept at room temperature until use

METHOD

- **Method/ guideline:** Guidelines for Screening Mutagenicity Testing of Chemicals (JAPAN) and OECD Test Guideline 471 and 472
- **Test type:** Reverse mutation assay
- **GLP:** Yes
- **Year:** 1996 (published year)
- **Species/Strain:** Salmonella typhimurium TA100, TA1535, TA98, TA1537 Escherichia coli WP2uvrA
- **Metabolic activation:** S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone
- **Statistical methods:** No statistic analysis

REMARKS FIELD FOR TEST CONDITIONS

Study Design:

- **Concentration:** -S9 mix: 0, 313, 625, 1250, 2500 and 5000 ug/plate
  +S9 mix: 0, 313, 625, 1250, 2500 and 5000 ug/plate
- **Number of replications:** 2
- **Plates/tests:** 3
- **Procedure:** Plate incorporation method
- **Solvent:** Water for injection
- **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 (five strains)

RESULTS

- **Cytotoxic concentration:**

  Toxicity was not observed up to 5000 ug/plate in the five strains with and without S9 mix.

- **Precipitation concentration:**

  Precipitation was not observed at any concentration with and without S9 mix.

- **Genotoxic effects:**

	+	?	-
With metabolic activation:	[ ]	[ ]	[X]
Without metabolic activation	[ ]	[ ]	[X]

REMARKS FIELD FOR RESULTS

CONCLUSIONS

Bacterial gene mutation is negative with and without metabolic activation.

DATA QUALITY

- **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan)

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals, 4, 449-450 (1996)

GENERAL REMARKS

GENETIC TOXICITY IN VITRO (NON-BACTERIL IN VITRO TEST)

TEST SBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No.126-33-0)
- Remarks: Source: Produced by Shinn Nippon Rika, Lot No.8050074
  Purity: above 99.9%

METHOD

- **Method/guideline:** Guideline for Screening Mutagenicity Testing of Chemicals (Japan)
- **Test type:** Chromosome aberration test
- **GLP:** Yes
- **Year:** 1996 (published year)
- **Species/ Strain:** CHL/IU cell
- **Metabolic activation:** S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone
- **Statistical methods:** Fisher's exact analysis

REMRKS FIELD FOR TEST CONDITIONS

Study Design:

For continuous treatment, cells were treated for 24 or 48 hrs without S9 mix.
For short-term treatment, cells were treated for 6 hrs with and without S9 mix and cultivated with fresh media for 18 hrs.

- **Concentration:**
  -S9 mix(continuous treatment): 0, 0.30, 0.60, 1.2 mg/ml
  -S9 mix(short-term treatment): 0, 0.30, 0.60, 1.2 mg/ml
  +S9 mix(short-term treatment): 0, 0.30, 0.60, 1.2 mg/ml
- **Plates/test:** 2
- **Solvent:** Water for injection
- **Positive controls:** Mitomycin C for continuous treatment
  Cyclophosphamide for short-term treatment

RESULTS

- **Cytotoxic concentration:**

  50 % Growth inhibition was not observed at any concentration with or without S9 mix.

- **Genotoxic effcts:**

	clastogenicity			polyploidy		
	+	?	-	+	?	-
With metabolic activation:	[ ]	[ ]	[X]	[ ]	[ ]	[X]
Without metabolic activation	[ ]	[ ]	[X]	[ ]	[ ]	[X]

REMARKS FIELD FOR RESULTS

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

CONCLUSIONS

Chromosomal aberration in CHL/IU cells is negative with and without metabolic activation.

DATA QUALITY

- **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan)

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 4, 451-453 (1996)

GENERAL RENARKS

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

TEST SUBSTANCE

- Tetrahydrothiophene-1,1-dioxide (CAS No. 126-33-0)
- Remarks: Source: Produced by Sumitomo Seika Chemicals, Lot No. 20802
  Purity: 97.3%, kept at room temperature until use

METHOD

- **Method／guideline:** OECD TG 421
- **Test Type:** OECD Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1999 (published year)
- **Species:** Rat
- **Strain:** Crj: CD(SD) IGS
- **Route of administration:** Oral (by gavage)
- **Dose /concentration levels:** 0, 60, 200, 700 mg/kg/day (in Water)
- **Sex:** Male & Female
- **Exposure period:** Male; for 49 days
  Female; for 41-50 days from 14 days prior to mating
  to the 3 day of lactation
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** none
- **Terminal killing:** Males; day 50
  Females; day 4 of lactation
- **Statistical methods:** Dunnett's (following to Bartlett or Kruskal-Wallis analysis)
  and Chi square test

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- **Age at study initiation:** 10 week old for both sexes
- **Weight at study initiation:**355-379 g for males, 209-225 g for females
- **No. of animals per sex per dose:** 12 per sex per dose group

Study Design:
The animals were sacrificed on the day 4 of lactation for females. Females with no copulation were killed on the final day of mating period. Females with no delivery were killed on the day 25 of pregnancy.

- **Vehicle:** Water
- **Satellite groups and reason they were added:** none
- **Mating procedure:** Male / female per cage; 1/1, length of cohabitation; at most 14 days, until proof of copulation (formation of vaginal plug or sperm detection in vagina)
- **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
  Microscopic: control & 700 mg/kg group/ testis, epididymis, ovary
  Pups: full macroscopic examinations on all pups
- **Parameters assessed during study:**
  Body weight and food consumption for males were determined twice a week, and for females, body weight and food consumption were determined twice a week prior to mating and in principle, once a week for gestation and lactation period. estrus cycle daily until successful day of copulation, No. of pairs successful copulation, copulation index (No. of pairs with successful copulation/No of pairs mated x 100), paring days until copulation, No. of pregnant females, fertility index=(No. of pregnant animals/ No. of pairs with successful copulation x 100), No. of corpora lutea, No. of implantation sites, implantation index (No. of implantation sites/No. of corpora lutea x 100), No. of living pregnant females, No. of pregnant females with parturition, gestation length, No. of pregnant females with live pups on day 0, gestation index(No. of females with live pups/No. of living pregnant females x 100), No. of pregnant females with live pups on day 4 , delivery I index(No. of pups born/ No. of implantation sites x 100), No. of pups alive on day 0 of lactation, live birth index(No. of live pups on day 0/ No. of pups born x 100), sex ratio(Total No. of male pups/ Total No. of female pups), No. of pups alive on day 4 of lactation, viability index(No. of live pups on day 4 / No. of live pups on day 0 x 100), body weight of live pups(on day 0 and 4)

RESULTS

- **NOAEL for reproductive performance:** 700 mg/kg/day for males,
  200 mg/kg /day for females

- **NOAEL: pups toxicity:**
  60 mg/kg/day

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

- **Pups data with dose level (with NOAEL value):**
  Birth index, live birth index, number of pups on days 0 and 4 of lactation, viability index, and body weights of both sexes on days 0 and 4 of lactation were decreased, and 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.

Reproductive parameters

Dose level(mg/kg)	0	60	200	700
No. of estrus cases(14 days)	3.3±0.5	3.3±0.5	3.2±0.4	2.2±0.9**
No. of dams	11	12	10	10
Birth index (%)	96.3±6.5	95.8±4.8	90.5±5.1*	71.6±26.2**
Dead pups on day 0	0.3±0.5	0.2±0.4	0.2±0.4	3.6±4.4**
Live birth index(%)	98.1±3.3	98.8±2.8	98.7±2.8	75.9±26.2**
Live pups on day 4	14.8±1.8	15.0±1.9	13.7±1.3	4.0±5.6**
Viability index(%)	99.5±1.8	100.0±0.0	97.3±1.3	29.2±40.4**
Body weight				
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±0.84	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)

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

REMARKS FIELD FOR RESULTS

- **Mortality and day of death:** One male and one female in the 700 mg/kg group died
- **General toxic signs:** Soiled fur for both sexes and soft stool for males in the 700 mg/kg group were noted.
  Body weight: Suppression of body weight gain in males, and in females during the pre-mating period in the 700 mg/kg was observed.
  Food consumption: Decrease of food consumption in males , and in females during the pre-mating and lactation period in the 700mg/kg was observed.
- **Pups data:** Grossly visible abnormalities : no significant effect was observed.

CONCLUSIONS

Toxic effects for female parent and pups are effects on reproductive parameters, such as decrease of the number of estrus cases and increase of dams losing all of their pups. With regards to pups, toxicity are effects on developmental parameters including number of pups, viability index, stillbirth and body weight.

The NOAELs are considered to be 200 mg/kg for maternal parents and 60 mg/kg for pups.

DATA QUALITY

- **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Nihon Bioresearch Inc. Hashima Laboratory (JAPAN)

REFERENCES(Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Envirnmental Chemicals 7, 471-481 (1999)

GENERAL REMARKS

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

a METI, Japan (1999) Ministry of Economy, Trade and Industry (former MITI), Test No.80132K conducted by Chemicals Evaluation and Research Institute, Japan (former CITI , Japan) unpublished data.

b Sumika Technoservice Corporation (2002a)

c Sumika Technoservice Corporation (2002b)

d METI, Japan (1975) Ministry of Economy, Trade and Industry (former MITI), conducted by Chemicals Evaluation and Research Institute, Japan (former CITI , Japan) unpublished data.

e METI, Japan (1977) Ministry of Economy, Trade and Industry (former MITI), conducted by Chemicals Evaluation and Research Institute, Japan (former CITI , Japan) unpublished data.

f EA, Japan (1999), The Environment Agency, Ecotoxicology testing report (unpublished), Test No. 92060 conducted by Chemical Evaluation and Research Institute, Japan (former CITI, Japan)

g EA, Japan (1999), The Environment Agency, Ecotoxicology testing report (unpublished), Test No. 92058 conducted by Chemical Evaluation and Research Institute, Japan (former CITI, Japan)

h EA, Japan (1999), The Environment Agency, Ecotoxicology testing report (unpublished), Test No. 92057 conducted by Chemical Evaluation and Research Institute, Japan (former CITI, Japan)

i EA, Japan (1999), The Environment Agency, Ecotoxicology testing report (unpublished), Test No. 92059 conducted by Chemical Evaluation and Research Institute, Japan (former CITI, Japan)