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Appendix I

I U C L I D

Data Set

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

Status :
Memo :

Printing date : 14.11.2006
Revision date : 25.10.2006
Date of last update : 14.11.2006

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Chapter (profile) : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
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 APPLICANT AND COMPANY INFORMATION

Type : manufacturer
Name : Chevron Phillips Chemical Company LP
Contact person :
Date :
Street : 10001 Six Pines Drive
Town : 77380 The Woodlands, TX
Country : United States
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR**1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION****1.1.2 SPECTRA****1.2 SYNONYMS AND TRADENAMES**

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

3-Sulfolene

butadiene sulfone

Sulfolene

1.3 IMPURITIES

1. General Information

Id 77-79-2
Date 14.11.2006

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1. General Information

Id 77-79-2
Date 14.11.2006

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2. Physico-Chemical Data

Id 77-79-2

Date

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 (9) (28)

Value : = 17.4 °C
Sublimation :
Method : other: EPIWIN v3.10
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 (35)

2.2 BOILING POINT

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
30.10.2006 (9) (23)

Decomposition : yes
Method : other: Decomposition
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : Temperature (deg F) / % Decomposition (wt%/hour) / Log (Dec. Rate)

158 / 0.1 / -1.0000
176 / 0.8 / -0.0969
194 / 2.6 / 0.4150

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212 / 8.4 / 0.9243
230 / 25.0 / 1.3979
Source : Chevron Phillips Chemical Co. LP
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
07.11.2006 (2)

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
26.10.2006 (35)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .013 hPa at 25 °C
Decomposition :
Method : Directive 92/69/EEC, A.4
Year : 2006
GLP : yes
Test substance : other TS

Result : The vapour pressure of Sulfolene was determined to be 1.3 Pa at 25 deg C based on the following results:

Run / Log10 [Vp(25degC)]
1 / 0.098
2 / 0.106
3 / 0.114
4 / 0.105
5 / 0.100
6 / 0.099
7 / 0.100

Mean: 0.103

Vapour Pressure: 1.268 Pa

Source : Chevron Phillips Chemical Company LP, 2006. Sulfolene (CAS number 77-79-2): Determination of Vapour Pressure. Draft Report. Study performed by SafePharm Laboratories, Shardlow, Derbyshire, UK.
Test condition : METHOD

Vapour pressure was determined using a vapour pressure balance with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25 deg C.

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CALCULATION

Vapour pressure (Pa) = mass difference (kg) X acceleration due to gravity (9.813 m/s²) / area of the orifice (7.06858E-6 m²)

Vapour pressure is related to temperature by the following equation:

$\text{Log}_{10}[\text{Vp(Pa)}] = \text{slope} / \text{temperature (K)} + \text{intercept}$

The vapour pressure of the sample was measured over a range of temperatures to enable extrapolation to 298.15 K.

Test substance : Sulfolene (CAS No 77-79-2)
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
07.11.2006 (29)

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
07.11.2006 (35)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = -.8 at °C
pH value :
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 hydrophobicity 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:

2. Physico-Chemical Data

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Date

Test substance	: Sittingbourne Research Centre, Sittingbourne, Kent.
Reliability	: Sulfolene obtained from Shell Chemicals U.K. Ltd. Sample contained 7% isopropyl alcohol.
Flag	: (1) valid without restriction
26.10.2006	: Critical study for SIDS endpoint (3) (10) (37)
Partition coefficient	:
Log pow	: < 1 at °C
pH value	:
Method	: other (measured): revers-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 hydrophobicity 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 (<i>Salmo gairdneri</i> , <i>Daphnia magna</i> , and <i>Selenastrum capricornutum</i>), 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 (8) (37)
26.10.2006	
Partition coefficient	:
Log pow	: = -.45 at °C
pH value	:
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 (35)
26.10.2006	

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	: Water
Value	: = 5.9 other: wt% at 25 °C
pH value	:
concentration	: at °C
Temperature effects	:
Examine different pol.	:
pKa	: at 25 °C
Description	:

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Date

Stable :
Deg. product :
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
26.10.2006 (9)

Solubility in : Water
Value : = 287900 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : very soluble (> 10000 mg/L)
Stable :
Deg. product :
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
26.10.2006 (35)

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 DISSOCIATION CONSTANT

2. Physico-Chemical Data

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Date 14.11.2006

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3. Environmental Fate and Pathways

Id 77-79-2

Date

3.1.1 PHOTODEGRADATION

Type : other
Light source :
Light spectrum : nm
Relative 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

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3.1.2 STABILITY IN WATER

Remark : Based on the chemical structure, sulfolene is not expected to undergo abiotic hydrolysis in the environment.

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3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other: air - water - soil - sediment
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: 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:

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Result : 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)
: 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
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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 : 20 mg/l related to Test substance related to
Contact time : 28 day(s)
Degradation : = 2 (±) % after 28 day(s)
Result : other: not readily biodegradable
Control substance : Benzoic acid, sodium salt
Kinetic : 5 day(s) > 50 %
15 day(s) > 75 %
Deg. product : not measured
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO₂ 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.

3. Environmental Fate and Pathways

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- 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 Flag** : (1) valid without restriction
: Critical study for SIDS endpoint
- 30.10.2006 (38)
- Type** : aerobic
- Inoculum** : other: Micro-organisms were obtained from Canterbury Sewage Works and prepared according to the prescribed methods for this test.
- Concentration** : 3 mg/l related to Test substance related to
- Contact time** : 28 day(s)
- Degradation Result** : = 0 (±) % after 28 day(s)
: other: Not readily biodegradable
- Control substance** : Benzoic acid, sodium salt
- Kinetic** : 5 day(s) > 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

3. Environmental Fate and Pathways

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Canterbury Sewage Works and prepared according to the prescribed methods for this test.

CONTROLS

Positive control: Sodium Benzoate

Control: Mineral medium

Blank: Microbial inoculum

Test substance : INTERMEDIATES/DEGRADATION PRODUCTS: Not identified.
: Approximately 90% sulfolene (2,5-dihydrothiophene 1,1-dioxide) and 7% isopropyl alcohol.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

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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.

Result : Calculated Koc using EPIWIN v 3.10 (PCKOC Program v 1.66)
: Estimated Log BCF = 0.500
BCF = 3.162

Source : Estimated Log Koc = 1.3343

Test substance : Estimated Koc = 21.59

Reliability : EPI Suite v 3.10

30.10.2006 : Thiophene, 2,5-dihydro-, 1,1-dioxide or Sulfolene (CAS Number 77-79-2).

Reliability : (2) valid with restrictions

30.10.2006

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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
 LC50 : = 940
 Limit test :
 Analytical monitoring : no
 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

Source : Mortality of Controls: 0
 : 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 CaCO₃
- 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

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4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

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

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Test condition

: Sittingbourne Research Centre, Sittingbourne, Kent.
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:

NaHCO₃ 192 mg/l
CaSO₄*2H₂O 120 mg/l
MgSO₄ 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

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4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)

Endpoint : growth rate

Exposure period : 4 day(s)

Unit : mg/l

EC50 : > 1000

Limit test :

Analytical monitoring : no data

Method : other

Year : 1983

GLP : no

Test substance : other TS

Method : Comparable 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:

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- 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-sterilized 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 CaCO₃
- 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 Flag** : (1) valid without restriction
: Critical study for SIDS endpoint

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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 SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 2876.1 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 55
Vehicle : no data
Doses :
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, lacrimation, 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.,

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Test condition	: Vienna Virginia. : 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
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5.1.2 ACUTE INHALATION TOXICITY

Type	: LC50
Value	:
Species	: rat
Strain	: Wistar
Sex	: male/female
Number of animals	: 10
Vehicle	: no data
Doses	:
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 (measured value not stated) 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

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Source	: seen in control rats, and absence of signs of pulmonary irritation lead to conclusion that microscopic findings are not treatment induced. : 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	: 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 accommodate 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 hanging 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.
Test substance	: Approximately 99% sulfolene (2,5-dihydrothiophene 1,1-dioxide) and 0.9% isopropyl alcohol
Reliability Flag	: (1) valid without restriction : Critical study for SIDS endpoint
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5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : other: undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle :
PDII : 0
Result : not irritating
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 gauze patch, secured with transparent 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

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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

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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
Vehicle :
Result : irritating
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

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Date 14.11.2006

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 fluorescein 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
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Species : rabbit
Concentration : other: normal saline slurry
Dose : 100 other: mg
Exposure time :
Comment :
Number of animals : 6
Vehicle :
Result : irritating
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

Source	: 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.
Test condition	: Phillips Petroleum Company Washed Primary Eye Irritation Study in Rabbits - Sulfolene - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia. : 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 fluorescein 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 30.10.2006	: (2) valid with restrictions

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5.3 SENSITIZATION

Type	: Guinea pig maximization test
Species	: guinea pig
Concentration	: 1 st . Induction 40 % occlusive epicutaneous 2 nd . Challenge 40 % occlusive epicutaneous 3 rd .
Number of animals	: 40
Vehicle	: other: acetone
Result	: not sensitizing
Classification	: not sensitizing
Method	: other
Year	: 1982

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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 response, 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 following 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 sacrificed with T-61.
- A skin section from the challenge site was fixed in 10% neutral buffered formalin, after the 72 hour challenge observation, for histopathologic 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
 30.10.2006

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5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : other: CrI:CD(SD)
Route of admin. : gavage
Exposure period : 28 days for males, 40-44 days for mated females, 52 days for females with no evidence of mating
Frequency of treatm. : Daily
Post exposure period :
Doses : Males: 25, 75, and 150 mg/kg/day
 Females: 10, 25, and 75 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL : = 25 mg/kg bw
Method : OECD combined study TG422
Year : 2006
GLP : yes
Test substance : other TS

Result : CLINICAL OBSERVATIONS AND SURVIVAL

-- Survival: One female rat in the 75 mg/kg/day group was found dead on lactation day 4, however, the death was not attributed to the test article due to the lack of remarkable clinical findings prior to death. All other males and females survived to the scheduled necropsies.

-- Clinical Observations: There were no test article-related clinical findings noted for surviving animals at any dosage level during the treatment period. Findings in the test article-treated groups, including hair loss and red or yellow material on various body surfaces, occurred in a manner that was not dose-related or at frequencies similar to those seen in the control group.

Clinical findings noted for recovery phase males and females, including hair loss, red material around the eye or nose, soft stool and/or a scabbed forelimb, were noted for single males and females in the 75 mg/kg/day group and were also noted for control group females. Therefore, none of the clinical findings noted during the recovery period were considered test article-related.

BODY WEIGHTS

-- Males, 150 mg/kg/day dose: Mean male body weight gains in the 150 mg/kg/day group were statistically significantly ($p < 0.01$) lower than the control group value during study days 0 through 7, but were similar to the control group throughout the remainder of the treatment period (study days 7-13, 13-21, and 21-28). As a result of the lower mean body weight gain noted in this group during the first week of the study, mean body weight gains were statistically significantly ($p < 0.01$) lower than the control group when the entire pre-mating (study days 0-13) and treatment (study days 0-28) periods were evaluated. However, during the recovery period (study days 28-42), mean body weight gain in this group was statistically significantly ($p < 0.01$) higher than the control group value. Mean body weights in the 150 mg/kg/day group were 5.3% to 6.0% lower than control group values during study days 7-28 as a result of the lower mean body weight gain during study days 0-7, and remained lower during the recovery period. The differences in mean body weight in the 150 mg/kg/day group were statistically significant ($p < 0.01$) relative to the control group beginning on study day 7 and continuing through the end of the treatment and recovery periods.

-- Males, 75 mg/kg/day dose: Mean male body weight gain was statistically significantly lower ($p < 0.01$) than the control group during study days 0-7, but was similar to the control group throughout the remainder of the treatment period (study days 7-13, 13-21, and 21-28). When the entire pre-mating period (study days 0-13) was evaluated, mean body weight gain in this group was slightly lower (not statistically significant) than the control group value, due primarily to the mean body weight loss noted during the first week of treatment. However, this lower mean body weight gain was not of sufficient magnitude to substantially affect mean body weights in this group.

-- Males, 25 mg/kg/day dose: Mean body weights and body weight gains were unaffected by test article administration.

-- Females, 75 mg/kg/day dose: Mean body weight gain was statistically significantly ($p < 0.01$) lower than the control group value during days 0-7, but was similar to the control group value during study days 7-13. This lower mean body weight gain in the 75 mg/kg/day group during the first week of treatment resulted in a statistically significantly lower ($p < 0.01$) mean body weight gain when the entire pre-mating period (study days 0-13) was evaluated. Mean body weight was statistically significantly lower than the control group on study day 7 ($p < 0.05$).

The lower mean body weight gain in the 75 mg/kg/day group during the first week of treatment did not result in a statistically significant mean body weight gain for recovery phase females when the entire treatment period (study days 0-42) was evaluated. Mean females body weights were up to 6.7% lower than control group values for the entire treatment period. However, a slightly higher (not statistically significant) mean body weight gain was noted in the 75 mg/kg/day group during the recovery period (study days 42-53), resulting in a mean body weight that was similar to the control group value on study day 53.

-- Females, 25 mg/kg/day dose: Mean body weights and body weight gains were similar to control group values during the pre-mating period.

-- Females, 10 mg/kg/day dose: Mean body weights and body weight gains were similar to control group values during the pre-mating period. Although a statistically significantly ($p < 0.05$) higher mean body weight gain was noted during study days 0-13 as a result of a higher mean body weight gain during study days 7-13, the increases were not considered test article-

related because no dose-relationship was apparent.

FOOD CONSUMPTION:

-- Males: Statistically significantly lower ($p < 0.01$) in the 75 and 150 mg/kg/day groups during study days 0-7 compared to the control group value. Mean food consumption in the 150 mg/kg/day group for the recovery phase group was similar to control group values during the remainder of the treatment period (study days 7-13, 13-21, and 21-28). However, the lower mean food consumption for the 75 and 150 mg/kg/day group males during the first week of treatment resulted in statistically significantly lower ($p < 0.05$ or $p < 0.01$) mean food consumption during both the pre-mating (study days 0-13) and treatment (study days 0-28; 150 mg/kg/day group only) periods. This pattern of lower food consumption corresponded to the effects observed on body weight gain in both groups. During the recovery period (study days 28-42), mean food consumption in the 150 mg/kg/day group was statistically significantly higher ($p < 0.01$) than the control group. This higher mean food consumption corresponded to the higher mean body weight gains in the 150 mg/kg/day group during the recovery period.

Mean pre-mating food consumption in the 25 mg/kg/day group males was similar to that in the control group throughout the study. No statistically significant differences were observed.

-- Females: Statistically significantly lower ($p < 0.01$) in the 75 mg/kg/day group during study days 0-7, but was similar to control group value during study days 7-13. The lower mean food consumption during the first week of treatment resulted in statistically significantly lower ($p < 0.01$) mean food consumption for the entire pre-mating (study days 0-13) period.

During the recovery period (study days 42-53), mean food consumption in the 75 mg/kg/day group was similar to control group value. However, the lower mean food consumption noted in this group during the first week of treatment resulted in statistically significantly lower ($p < 0.01$) mean food consumption for recovery phase females when the entire treatment period (study days 0-42) was evaluated. The only other statistically significant difference from the control group noted for recovery phase females in the 75 mg/kg/day group was slightly lower ($p < 0.05$; g/animal/day only) during study days 21-28.

Mean food consumption in the 10 and 25 mg/kg/day group females was generally similar to that in the control group throughout the pre-mating period. The only statistically significant ($p < 0.05$) difference was higher food consumption in the 10 mg/kg/day group during study days 7-13. No dose-response relationship was evident; therefore, this increase was not considered test article-related.

FUNCTIONAL OBSERVATIONAL BATTERY

-- Home cage, handling, open field, sensory, neuromuscular, and physiological observations evaluated on study day 28 (males) or lactation day 4 (females) were unaffected by test article administration at any dosage level.

-- Mean male hindlimb grip strength was slightly lower (not statistically significant) than the control group value at all dosage levels, and rotarod performance was lower (not statistically significant) at 25 and 75 mg/kg/day compared to the control group value. Since neither were decreased in a dose-related manner, no relationship to test article administration was apparent. Mean male forelimb grip strength in the 25, 75, and 150 mg/kg/day groups was not statistically significantly different from the control

group value. Mean grip strength (forelimb and hindlimb) for females in the test article-treated groups were similar to control group values; differences were not statistically significant.

LOCOMOTOR ACTIVITY

-- Total and ambulatory activity during the first three session intervals (0-15 minute, 16-30 minute, and 31-45 minute) and during the overall 60-minute test session were statistically significantly ($p=0.007$) increased in the 150 mg/kg/day group males. Overall total and ambulatory activity values in this group were 58.5% and 54.5%, respectively, higher than the control group. Although there was no effect on habituation for the 150 mg/kg/day males, the slight increases in motor activity were considered test article-related.

-- Locomotor activity patterns (total activity and ambulatory activity counts) were unaffected by test article administration for the 25 and 75 mg/kg/day dosed males and for the 10, 25, and 75 mg/kg/day dosed females when evaluated on study day 28 (males) or lactation day 4 (females). Differences from the control group were slight, not statistically significant and/or did not occur in a dose-related manner.

CLINICAL PATHOLOGY

-- The following alterations in hematology parameters were considered to be related to test article administration:

Data are presented in the following format: Parameter / 0 mg/kg Dose / 25 mg/kg Dose / 75 mg/kg Dose / 150 mg/kg Dose / Historical Control Mean (Range)

% Reticulocytes / 1.9 / 2.3 / 2.4 / 2.6* / 1.6 (0.0 - 8.3)
Absolute Reticulocytes (thous/uL) / 158.5 / 181.5 / 190.6 / 209.2* / 0.122 (0.000 - 0.623) mil/uL

* Values statistically significantly ($p<0.05$) different from control group values

Reticulocyte changes in males were considered test article-related due to a dose response. However, the alterations were of slight magnitude and within historical control range; therefore, they were not considered to be toxicologically significant or adverse. There were no histologic correlates.

There were no other test article-related effects on hematology data.

SERUM CHEMISTRY

There were no test article-related alterations in serum chemistry parameters. Mean serum phosphorus was statistically significantly ($p<0.05$ or $p<0.01$) higher in the 25 and 150 mg/kg/day male groups but was not considered to be test article-related because the values did not show a dose response.

URINALYSIS

Urinalysis revealed statistically significantly ($p<0.01$) higher mean urine pH in the 150 mg/kg/day male group (taken from recovery rats following the 28th dose). Although the pH alteration was considered test article-related, it was not considered adverse as the value fell within normal control values in the historical control database. In addition, the total volume of urine for recovery phase males in the 150 mg/kg/day group was increased by more than 2-fold compared to that of the control group. There were no other test article-related effects on urinalysis parameters.

ANATOMIC PATHOLOGY

-- Macroscopic Examinations: A distended cecum was noted for one female in the 75 mg/kg/day group found dead on lactation day 4; this death was not considered test article-related. No test article related internal findings were observed at the primary or recovery necropsies for males at 25, 75 and 150 mg/kg/day or for females at 10, 25 and 75 mg/kg/day. Macroscopic findings noted in test article-treated groups occurred in single animals, at a similar frequency to the control group and/or in a manner that was not dose related.

-- Organ Weights: Higher liver weights in males were considered to be test article-related at 150 mg/kg/day due to supportive microscopic changes of hepatocellular hypertrophy in the 150 mg/kg/day group males. Higher kidney weights in males were also considered to be test article-related at 150 mg/kg/day; hyaline droplets were noted in the 75 and 150 mg/kg/day group males.

Test Article-Related Liver and Kidney Weight Alterations in Males, Primary Necropsy: Data are presented in the following format: Dose (g/kg/day) / Absolute liver weight (g) / Relative liver weight to final body weight (g/100g) / Absolute kidney weight (g) / Relative kidney weight to final body weight (g/100g)

0 / 16.7 / 3.846 / 3.30 / 0.762
 25 / 18.07 / 3.991 / 3.45 / 0.766
 75 / 17.02 / 3.892 / 3.47 / 0.795
 150 / 17.60 / 4.204* / 3.63 / 0.867*

* Value statistically significantly ($p < 0.01$) different from control group values

At the recovery necropsy, there was no statistical difference in absolute mean organ weights between control and test article-treated group animals.

Brain weight (relative to final body weight) was statistically significantly ($p < 0.05$) higher for females in the 75 mg/kg/day group compared to the control group value at the scheduled necropsy. Brain and heart weights (relative to final body weight) were statistically significantly ($p < 0.05$ or $p < 0.01$) higher for males at 150 mg/kg/day compared to control group values at the recovery necropsy. These differences were considered to be a result of test article-related effects on final body weight. There were no other test article-related effects on organ weights.

-- Microscopic Examinations: Test article-related microscopic changes were present in the liver of males at 150 mg/kg/day and in the kidneys of males at 75 and 150 mg/kg/day. There were no test article-related microscopic changes in females.

Incidence of Selected Histopathologic Findings in Males, Primary Necropsy (12 tissues examined per group): Data are presented in the following format: Finding / 0 mg/kg/day / 25 mg/kg/day / 75 mg/kg/day / 150 mg/kg/day

Liver:

- Centrilobular hypertrophy / 0 / 0 / 0 / 8+
- Minimal / 0 / 0 / 0 / 5+
- Mild / 0 / 0 / 0 / 3+

Kidney:

- Basophilic tubules / 4 / 3 / 3 / 9+

- Minimal / 4 / 3 / 2 / 8+
- Mild / 0 / 0 / 1 / 1+
- Hyaline droplets / 2 / 0 / 8+ / 12+
- Minimal / 2 / 0 / 5+ / 1+
- Mild / 0 / 0 / 3+ / 11+

The liver of 8/12 males in the 150 mg/kg/day group had minimal to mild centrilobular hepatocellular hypertrophy that ranged in severity from minimal to mild (grades 1-2, respectively, on a 1-4 scale). The lesions were concentrated in the central region of the classical hepatic lobule and consisted of swollen hepatocytes.

Hyaline droplets, appearing as intracytoplasmic, brightly eosinophilic, partially refractile material, were present in proximal tubular epithelial cells of kidneys in 2/12, 0/12, 8/12 and 12/12 males in the control, 25, 75 and 150 mg/kg/day groups, respectively. Basophilic tubules, graded as minimal to mild, were present in the kidneys of 4/12, 3/12, 3/12 and 9/12 males in the same respective groups.

There were no test article-related changes in livers and kidneys examined from recovery males.

There were no other test article-related histologic changes. Remaining histologic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than administration of the test article. There was no test article-related alteration in the incidence, severity or histologic character of those incidental and spontaneous tissue alterations. Intracytoplasmic hyaline droplet formation in the renal proximal tubular cells of male rats is most often associated with accumulation of alpha 2 μ -globulin, which is specific to male rats. Renal tubular regeneration ("basophilic tubules") is commonly associated with alpha 2 μ -globulin nephropathy. Renal effects in male rats resulting from chemicals that cause alpha 2 μ -globulin accumulation are generally not expected to cause similar renal effects in humans (Hard, 1993). Hepatocellular centrilobular hypertrophy is considered a non-adverse adaptive response and is consistent with hepatic enzyme induction.

- Source** : Chevron Phillips Chemical Company LP, 2006. A Combined 28-Day Repeated Dose Oral Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of 3-Sulfolene in Rats, with Recovery - Audited Draft Report. Study performed by WIL Research Laboratories, LLC, Ashland, Ohio.
- Test condition** : TEST ANIMALS

CrL:CD(SD) rats from Charles River Laboratories. Animals were approximately 10 weeks old at the initiation of the study. Animal weights ranged from 331 to 369 g for males and 218 to 249 g for females on study day 0.

Animal Diet and Conditions: The basal diet used in the study was Certified Rodent LabDiet 5002 (PMI Nutrition International, LLC). Reverse osmosis-purified drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the acclimation period (10 days) and during the study. All rats were housed throughout the acclimation period and during the study in an environmentally controlled room. Mean daily temperature ranged from 21.3 to 21.7 deg C and mean daily relative humidity ranged from 41.1 to 59.2% during the study. A 12-hour light/12-hour dark photoperiod was provided. Air handling units provided a minimum of 10 fresh air changes per hour.

VEHICLE

100% Mazola corn oil stored at room temperature (exp. dates: 7 Dec 2006, 29 Dec 2006, or 7 March 2007).

TEST SUBSTANCE

Dosing formulations prepared at test article concentrations ranging from 2.5 to 37.5 mg 3-sulfolene/mL were analyzed to confirm test article concentration and the results met the SOP acceptance criteria for test article concentration in suspension formulations, i.e, the analyzed concentrations were within 85% to 115% of the target concentrations, with the following exception. The 37.5 mg/mL formulation prepared on 30 November 2005 for administration to males in Group 5 (150 mg/kg/day) was 79.6% of the target concentration. That formulation was reanalyzed and the next formulation scheduled to be dispensed was resuspended, sampled and analyzed the following day (1 December 2005). The results met the SOP requirement for concentration acceptability for suspension formulations.

ORGANIZATION OF TEST GROUPS, DOSAGE LEVELS, AND TREATMENT REGIMENS

The vehicle and test article formulations were administered orally by gavage once daily.

Males were dosed during the study days 0-27 (14 days prior to pairing through 1 day prior to scheduled euthanasia), for a total of 28 doses. At the end of the 28-day period, males assigned to the recovery groups remained on study for a 14-day recovery period without treatment.

Females were dosed during study days 0 through the day prior to euthanasia (14 days prior to pairing through laccation day 3) for a total of 40 to 44 doses. Females with no evidence of mating were dosed through the day prior to euthanasia to a total of 52 doses. Following 39 doses, females assigned to the recovery groups remained on study for a 14-day recovery period without treatment.

Dosage Volume: 4 mL/kg for all groups Individual dosages were based on the most recent recorded body weights. All animals were dosed at approximately the same time each day.

Number of Animals Dosed:

- Vehicle: 18 animals/sex
- 25 mg/kg/day (males): 12 animals
- 10 mg/kg/day (females): 12 animals
- 75 mg/kg/day (males): 12 animals
- 25 mg/kg/day (females): 12 animals
- 150 mg/kg/day (males): 18 animals
- 75 mg/kg/day (females): 18 animals

At the end of the study, 6 animals/sex in the control and high-dose groups remained on study for 14 days without treatment.

Dosage levels were selected based on the results of a 14-day pilot study.

PARAMETERS EVALUATED

All rats were observed twice daily (morning and afternoon) for moribundity and mortality. Detailed physical examinations were recorded weekly.

--BODY WEIGHTS: Recorded weekly. Female body weights were recorded weekly until evidence of copulation was observed. Following copulation, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17, and 20 and on lactation days 1 and 4.

-- FOOD CONSUMPTION: Recorded on the corresponding weekly body weight days until pairing. Food intake not recorded during the mating period. Once evidence of mating was observed, female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Following mating, food consumption for females with no evidence of mating and for all males was measured on a weekly basis until the scheduled euthanasia.

-- FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

---- Home Cage Observations: Posture, biting, convulsions/tremors, palpebral (eyelid) closure, feces consistency.

---- Handling Observations: Ease of removal from cage, ease of handling animal in hand, lacrimation/chromodacryorrhea, salivation, piloerection, fur appearance, palpebral closure, respiratory rate/character, eye prominence, mucous membranes/eye/skin color, red/crusty deposits, muscle tone.

---- Open Field Observations (Evaluated over a 2-minute observation period): Mobility, gait, rearing, arousal, convulsions/tremors, urination/defecation, grooming, gait score, bizarre/stereotypic behavior, backing, time to first step (seconds).

---- Sensory Observations: Approach response, touch response, startle response, tail pinch response, pupil response, eyeblink response, forelimb extension, hindlimb extension, air righting reflex, olfactory orientation.

---- Neuromuscular Observations: Hindlimb extensor strength, grip strength-hind and forelimb, hindlimb foot splay, rotarod performance.

---- Physiological Observations: Catalepsy, body weight, body temperature.

-- LOCOMOTOR ACTIVITY: Locomotor activity counts were recorded for 6 animals/sex/group following approximately 28 days of dose administration (males) and on lactation day 4 (females). Locomotor activity was measured automatically using the San Diego Instruments, Inc., Photobeam Activity System (San Diego Instruments, Inc., San Diego, California). Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills and ambulatory motor activity.

-- CLINICAL PATHOLOGY: Blood samples for clinical pathology evaluations (hematology and serum chemistry) were collected from 6 animals/sex/group at the scheduled necropsies. These animals were not fasted overnight prior to blood collection. Urine was collected from the 6 recovery phase rats/sex in the control and high-dose groups. The following parameters were evaluated:

---- Hematology: Total leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count (percent and absolute), differential leukocyte count (percent and absolute, neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell).

---- Serum Chemistry: Albumin, total protein, globulin (by calculation), albumin/globulin ratio (by calculation), total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate

aminotransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium.

---- Urinalysis: Bilirubin, color, clarity, glucose, ketones, leukocytes, microscopy of sediment, occult blood, pH, protein, specific gravity, total volume.

-- MACROSCOPIC EXAMINATIONS

----Unscheduled Death

----Scheduled Euthanasia: All surviving adults were euthanized by carbon dioxide inhalation.

---- Necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera. At the time of necropsy, the following tissues and organs were placed in 10% neutral-buffered formalin: Adrenal glands, aorta, bone with marrow (sternabrae), bone marrow smear (not taken from animal found dead, not placed in formalin, examined only if warranted), brain (forebrain, midbrain, hindbrain), coagulating glands, eyes with optic nerve (placed in Davidson's solution), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), heart, kidneys, exorbital lacrimal glands, liver (sections of 2 lobes), lungs (including bronchi, fixed by inflation with fixative), lymph node (mesenteric and mandibular), mammary gland (females only), ovaries and oviducts, pancreas, peripheral nerve (sciatic), pituitary gland, prostate gland, salivary gland (mandibular), seminal vesicles, skeletal muscle (rectur femoris), skin, spinal cord (cervical, thoracic and lumbar), spleen, testes with epididymides (fixed in Bouin's solution), thymus, thyroids, trachea, urinary bladder, uterus with vagina (uterus not taken from females found to be nonpregnant), all gross lesions.

---- Organ Weights: The following organs were weighted from all animals at the scheduled necropsies: Adrenal glands, brain, epididymides (weighed separately), heart, kidneys, liver, ovaries and oviducts, spleen, testes, thymus gland, thyroids with parathyroids. Absolute weights and organ to final body weight ratios were reported.

-- MICROSCOPIC EXAMINATIONS: Protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin, with the following exceptions. PAS staining was used for the testes and epididymides. The testes were fixed in Bouin's solution and embedded in paraffin. Microscopic examination was performed on all tissues listed above from all animals selected for the reproduction phase in the control and 150 mg/kg/day (males) or 75 mg/kg/day (females) groups at the scheduled necropsies, and from females that died or that failed to deliver. Based on the results of these evaluations, livers and kidneys from low- and mid-dosage group males, as well as from all recovery phase males, were also examined microscopically. Recovery phase females were not examined microscopically.

Because hyaline droplets were observed for male rats in the control, 75 and 150 mg/kg/day groups, immunohistochemical staining of the kidney tissue was performed for 5 male rats each in the control and 150 mg/kg/day groups in order to evaluate the presence of alpha-2u-globulin. The control rats selected included at least 1 animal observed microscopically with hyaline droplet formation. One tissue block from each male rat was processed. From each block, 2 sections of approximately 3-micron thickness were collected. One section was used as negative control, and the second section was incubated with the antibody. The evaluation of alpha-2u-globulin was performed via routine light microscopy.

STATISTICAL ANALYSIS

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation and the number of animals used to calculate the mean. In addition, percent change from control is presented for body weights, clinical pathology parameters and organ weights. Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, absolute and relative organ weights, clinical pathology values (excluding differential white cell counts other than lymphocytes and neutrophils), and FOB data values were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-treated groups to the control group. FOB parameter that yield scalar or descriptive data in the test article-treated groups were compared to the control group using Fisher's Exact test (Steel and Torrie, 1980).

For locomotor activity, total counts were analyzed by sex and session, with a repeated measure analysis of variance (RANOVA). Factors in the model included treatment group (TRT), time interval (TIME) and the interaction of time interval and treatment group (TRT*TIME). The SAS procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC) for first-order autoregressive homogeneous ([AR(1)]) and compound symmetric (CS) structures. The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dosage levels. The linear dose by time interaction (LinTrt*Time) was evaluated, and if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals by session only.

Nonmonotonic dose responses were evaluated whenever no significant linear trends were detected by TRT and/or TRT*TIME interaction was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted across the pooled time intervals of the entire session. These nonmonotonic dose response comparisons were conducted at the 0.01 significance level.

All statistical analyses were conducted using SAS version 8.2 (SAS Institute, Inc., 1999-2001) software. Total count locomotor activity data were analyzed by BioSTAT Consultants, Inc., Portage, Michigan.

Ambulatory counts measured in the locomotor activity assessment were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup variance. If significant differences were indicated by the ANOVA, Dunnett's test (Dunnett, 1964) was used to compare the control and test article-treated groups.

**Test substance
Conclusion**

- : 3-sulfolene (CAS# 77-79-2, 98.9% pure)
- : Based on the lower mean body weights, body weight gains and food consumption at 75 mg/kg/day (males and females) and 150 mg/kg/day (males), a dosage level of 25 mg/kg/day was considered to be the NOAEL for systemic toxicity.

**Reliability
Flag**

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

09.11.2006

(7) (11) (22) (27) (31) (39)

5. Toxicity

Id 77-79-2

Date 14.11.2006

Type :
Species : rat
Sex : male/female
Strain : Osborne-Mendel
Route of admin. : gavage
Exposure period : 6 weeks
Frequency of treatm. : 5 consecutive days per week for 6 weeks
Post exposure 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

5. Toxicity

Id 77-79-2

Date 14.11.2006

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 Flag : (2) valid with restrictions
30.10.2006 : Critical study for SIDS endpoint (34)

Type :
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 6 weeks
Frequency of treatm. : 5 consecutive days per week for 6 weeks
Post exposure 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 \leq 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 \leq 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 Flag : (2) valid with restrictions
: Critical study for SIDS endpoint

30.10.2006 (34)

5.5 GENETIC TOXICITY 'IN VITRO'

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

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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.

Source : Sulfolene is considered not to be mutagenic in this test system. (author)
: 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 x10⁵ 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: Etylmethanesulfonate

- With Activation

Negative: Medium, Dimethylsulfoxide

Positive: 3-Methylcholanthrene.

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

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Reliability : 100% (Hazleton); Solubility was 100 mg/ml in cell culture medium.
Flag : (1) valid without restriction
08.11.2006 : Critical study for SIDS endpoint (15)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains: 1535, 1537, 1538, TA98, and TA100
Test concentration : 10,000; 3,333.3; 1,111.1; 370.4; and 123.5 ug/plate
Cycotoxic concentr. :
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

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TA98 - 33, 28, 29
TA100 - 90, 103, 104

Source : GENOTOXIC EFFECTS:
Negative with and without metabolic activation.
: 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

Test substance : METABOLIC ACTIVATION: Aroclor-induced rat liver microsomal fraction.
: 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
14.11.2006 (17)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary Cells, CCL61
Test concentration : 1000, 334, 100, 34, 10 ug/ml
Cycotoxic concentr. :
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)

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Date 14.11.2006

- 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 Flag** : (1) valid without restriction
08.11.2006 : Critical study for SIDS endpoint (14)
- Type** : Chromosomal aberration test
- System of testing** : Chinese hamster ovary cells
- Test concentration** : 0, 368, 1110, and 3680 ug/ml
- Cytotoxic concentr.** :
- 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

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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

Source
Test condition

Results for Sulfolene were negative both with and without activation.
: Loveday et al., 1990.
: 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 Flag : (2) valid with restrictions
: Critical study for SIDS endpoint

30.10.2006 (24) (25)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

Species : rat

Sex : male/female

Strain : Osborne-Mendel

Route of admin. : gavage

Exposure period : 60 to 78 weeks

Frequency of treatm. : Five consecutive days per week

Post exposure 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).
Remark	: Endpoints Examined: Mortality, weight changes, pathology/tumor incidence 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.
Result	: Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 3 sulfolene to Osborne-Mendel rats. 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 ($P < 0.001$).

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- 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 (1) (4) (5) (21) (30) (33) (34)

Species : mouse

Sex : male/female

Strain : B6C3F1

Route of admin. : gavage

Exposure period : 60-78 weeks

Frequency of treatm. : five consecutive days per week

Post exposure 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).

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

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

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.

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

Reliability : (2) valid with restrictions

30.10.2006

(1) (4) (5) (21) (30) (33) (34)

5.8.1 TOXICITY TO FERTILITY

Type : other: Combined repeat dose toxicity study with reproduction/developmental toxicity screening test

Species : rat

Sex : male/female

Strain : other: CrI:CD(SD)

Route of admin. : gavage

Exposure period : 28 days for males, 40-44 days for mated females, 52 days for females with no evidence of mating

Frequency of treatm. : Daily

5. Toxicity

Id 77-79-2

Date

Premating exposure period

Male	:	14 days
Female	:	14 days
Duration of test	:	28 days for males, 40-44 days for mated females, 52 days for females with no evidence of mating
No. of generation studies	:	1
Doses	:	Males: 25, 75, and 150 mg/kg/day Females: 10, 25, and 75 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 75 mg/kg bw
NOAEL F1 offspring	:	= 25 mg/kg bw
Method	:	OECD Guide-line 422
Year	:	2006
GLP	:	yes
Test substance	:	other TS

Result : CLINICAL OBSERVATIONS AND SURVIVAL

-- Survival: One female rat in the 75 mg/kg/day group was found dead on lactation day 4, however, the death was not attributed to the test article due to the lack of remarkable clinical findings prior to death. All other males and females survived to the scheduled necropsies.

-- Clinical Observations: There were no test article-related clinical findings noted for surviving animals at any dosage level during the treatment period.

BODY WEIGHT, MALES

-- 150 mg/kg/day dose: Mean male body weight gains in the 150 mg/kg/day group were statistically significantly ($p < 0.01$) lower than the control group value during study days 0 through 7, but were similar to the control group throughout the remainder of the treatment period (study days 7-13, 13-21, and 21-28). As a result of the lower mean body weight gain noted in this group during the first week of the study, mean body weight gains were statistically significantly ($p < 0.01$) lower than the control group when the entire pre-mating (study days 0-13) and treatment (study days 0-28) periods were evaluated. However, during the recovery period (study days 28-42), mean body weight gain in this group was statistically significantly ($p < 0.01$) higher than the control group value. Mean body weights in the 150 mg/kg/day group were 5.3% to 6.0% lower than control group values during study days 7-28 as a result of the lower mean body weight gain during study days 0-7, and remained lower during the recovery period. The differences in mean body weight in the 150 mg/kg/day group were statistically significant ($p < 0.01$) relative to the control group beginning on study day 7 and continuing through the end of the treatment and recovery periods.

-- 75 mg/kg/day dose: Mean male body weight gain was statistically significantly lower ($p < 0.01$) than the control group during study days 0-7, but was similar to the control group throughout the remainder of the treatment period (study days 7-13, 13-21, and 21-28). When the entire pre-mating period (study days 0-13) was evaluated, mean body weight gain in this group was slightly lower (not statistically significant) than the control group value, due primarily to the mean body weight loss noted during the first week of treatment. However, this lower mean body weight gain was not of sufficient magnitude to substantially affect mean body weights in this group.

-- 25 mg/kg/day dose: Mean body weights and body weight gains were unaffected by test article administration.

BODY WEIGHT, FEMALES

-- Pre-Mating, Mating and Recovery:

---- 75 mg/kg/day dose: Mean body weight gain was statistically significantly ($p < 0.01$) lower than the control group value during days 0-7, but was similar to the control group value during study days 7-13. This lower mean body weight gain in the 75 mg/kg/day group during the first week of treatment resulted in a statistically significantly lower ($p < 0.01$) mean body weight gain when the entire pre-mating period (study days 0-13) was evaluated. Mean body weight was statistically significantly lower than the control group on study day 7 ($p < 0.05$).

The lower mean body weight gain in the 75 mg/kg/day group during the first week of treatment did not result in a statistically significant mean body weight gain for recovery phase females when the entire treatment period (study days 0-42) was evaluated. Mean females body weights were up to 6.7% lower than control group values for the entire treatment period. However, a slightly higher (not statistically significant) mean body weight gain was noted in the 75 mg/kg/day group during the recovery period (study days 42-53), resulting in a mean body weight that was similar to the control group value on study day 53.

---- 25 mg/kg/day dose: Mean body weights and body weight gains were similar to control group values during the pre-mating period.

---- 10 mg/kg/day dose: Mean body weights and body weight gains were similar to control group values during the pre-mating period. Although a statistically significantly ($p < 0.05$) higher mean body weight gain was noted during study days 0-13 as a result of a higher mean body weight gain during study days 7-13, the increases were not considered test article-related because no dose-relationship was apparent.

-- Gestation:

---- 75 mg/kg/day dose: Mean body weights and body weight gains were generally similar to those in the control group throughout gestation (days 0-4, 4-7, 7-11, 11-14, 14-17, 17-20 and 0-20); there were no statistically significant differences.

---- 25 mg/kg/day and 10 mg/kg/day doses: Mean body weights and body weight gains were similar to control group values during gestation.

-- Lactation:

---- 75 mg/kg/day dose: Mean body weight gains were generally similar to the control group during lactation days 1-4; differences were not statistically significant. Mean body weights were 8.6% and 6.9% lower than the control group values on lactation days 1 and 4, respectively; the difference on lactation day 1 was statistically significant ($p < 0.05$). The lower mean body weights noted in the 75 mg/kg/day group on lactation days 1 and 4 were attributed primarily to the lower mean body weight gain in this group during the first week of treatment.

---- 25 mg/kg/day and 10 mg/kg/day doses: Mean body weights and body weight gains were unaffected by test article administration on lactation days 1 and 4.

FOOD CONSUMPTION:

-- Males: Statistically significantly lower ($p < 0.01$) in the 75 and 150 mg/kg/day groups during study days 0-7 compared to the control group

value. Mean food consumption in the 150 mg/kg/day group for the recovery phase group was similar to control group values during the remainder of the treatment period (study days 7-13, 13-21, and 21-28). However, the lower mean food consumption for the 75 and 150 mg/kg/day group males during the first week of treatment resulted in statistically significantly lower ($p<0.05$ or $p<0.01$) mean food consumption during both the pre-mating (study days 0-13) and treatment (study days 0-28; 150 mg/kg/day group only) periods. This pattern of lower food consumption corresponded to the effects observed on body weight gain in both groups. During the recovery period (study days 28-42), mean food consumption in the 150 mg/kg/day group was statistically significantly higher ($p<0.01$) than the control group. This higher mean food consumption corresponded to the higher mean body weight gains in the 150 mg/kg/day group during the recovery period.

Mean pre-mating food consumption in the 25 mg/kg/day group males was similar to that in the control group throughout the study. No statistically significant differences were observed.

-- Females: Pre-Mating, Mating and Recovery: Statistically significantly lower ($p<0.01$) in the 75 mg/kg/day group during study days 0-7, but was similar to control group value during study days 7-13. The lower mean food consumption during the first week of treatment resulted in statistically significantly lower ($p<0.01$) mean food consumption for the entire pre-mating (study days 0-13) period.

During the recovery period (study days 42-53), mean food consumption in the 75 mg/kg/day group was similar to control group value. However, the lower mean food consumption noted in this group during the first week of treatment resulted in statistically significantly lower ($p<0.01$) mean food consumption for recovery phase females when the entire treatment period (study days 0-42) was evaluated. The only other statistically significant difference from the control group noted for recovery phase females in the 75 mg/kg/day group was slightly lower ($p<0.05$; g/animal/day only) during study days 21-28.

Mean food consumption in the 10 and 25 mg/kg/day group females was generally similar to that in the control group throughout the pre-mating period. The only statistically significant ($p<0.05$) difference was higher food consumption in the 10 mg/kg/day group during study days 7-13. No dose-response relationship was evident; therefore, this increase was not considered test article-related.

-- Females, Gestation: No test article-related effects on mean maternal food consumption were observed at any dosage level during gestation. None of the differences from the control group were statistically significant.

-- Females, Lactation: No test article-related effects on mean maternal food consumption were observed at any dosage level during lactation days 1-4. None of the differences from the control group were statistically significant.

FUNCTIONAL OBSERVATIONAL BATTERY

No statistically significant treatment-related effects were detected.

LOCOMOTOR ACTIVITY

-- Total and ambulatory activity during the first three session intervals (0-15 minute, 16-30 minute, and 31-45 minute) and during the overall 60-minute test session were statistically significantly ($p=0.007$) increased in the 150 mg/kg/day group males. Overall total and ambulatory activity values in this

group were 58.5% and 54.5%, respectively, higher than the control group. Although there was no effect on habituation for the 150 mg/kg/day males, the slight increases in motor activity were considered test article-related.

-- Locomotor activity patterns (total activity and ambulatory activity counts) were unaffected by test article administration for the 25 and 75 mg/kg/day dosed males and for the 10, 25, and 75 mg/kg/day dosed females when evaluated on study day 28 (males) or lactation day 4 (females). Differences from the control group were slight, not statistically significant and/or did not occur in a dose-related manner.

CLINICAL PATHOLOGY

-- HEMATOLOGY

The following alterations in hematology parameters were considered to be related to test article administration:

Data are presented in the following format: Parameter / 0 mg/kg Dose / 25 mg/kg Dose / 75 mg/kg Dose / 150 mg/kg Dose / Historical Control Mean (Range)

% Reticulocytes / 1.9 / 2.3 / 2.4 / 2.6* / 1.6 (0.0 - 8.3)
Absolute Reticulocytes (thous/uL) / 158.5 / 181.5 / 190.6 / 209.2* / 0.122 (0.000 - 0.623) mil/uL

* Values statistically significantly ($p < 0.05$) different from control group values

Reticulocyte changes in males were considered test article-related due to a dose response. However, the alterations were of slight magnitude and within historical control range; therefore, they were not considered to be toxicologically significant or adverse. There were no histologic correlates.

There were no other test article-related effects on hematology data.

-- SERUM CHEMISTRY

There were no test article-related alterations in serum chemistry parameters.

-- URINALYSIS

Urinalysis revealed statistically significantly ($p < 0.01$) higher mean urine pH in the 150 mg/kg/day male group (taken from recovery rats following the 28th dose). Although the pH alteration was considered test article-related, it was not considered adverse as the value fell within normal control values in the historical control database. In addition, the total volume of urine for recovery phase males in the 150 mg/kg/day group was increased by more than 2-fold compared to that of the control group. There were no other test article-related effects on urinalysis parameters.

ANATOMIC PATHOLOGY

-- MACROSCOPIC EXAMINATIONS

A distended cecum was noted for one female in the 75 mg/kg/day group found dead on lactation day 4; this death was not considered test article-related. No test article related internal findings were observed at the primary or recovery necropsies for males at 25, 75 and 150 mg/kg/day or for females at 10, 25 and 75 mg/kg/day.

-- ORGAN WEIGHTS

Higher liver weights in males were considered to be test article-related at 150 mg/kg/day due to supportive microscopic changes of hepatocellular hypertrophy in the 150 mg/kg/day group males. Higher kidney weights in males were also considered to be test article-related at 150 mg/kg/day; hyaline droplets were noted in the 75 and 150 mg/kg/day group males.

Test Article-Related Liver and Kidney Weight Alterations in Males, Primary Necropsy: Data are presented in the following format: Dose (g/kg/day) / Absolute liver weight (g) / Relative liver weight to final body weight (g/100g) / Absolute kidney weight (g) / Relative kidney weight to final body weight (g/100g)

0 / 16.7 / 3.846 / 3.30 / 0.762
 25 / 18.07 / 3.991 / 3.45 / 0.766
 75 / 17.02 / 3.892 / 3.47 / 0.795
 150 / 17.60 / 4.204* / 3.63 / 0.867*

* Value statistically significantly ($p < 0.01$) different from control group values

At the recovery necropsy, there was no statistical difference in absolute mean organ weights between control and test article-treated group animals.

Brain weight (relative to final body weight) was statistically significantly ($p < 0.05$) higher for females in the 75 mg/kg/day group compared to the control group value at the scheduled necropsy. Brain and heart weights (relative to final body weight) were statistically significantly ($p < 0.05$ or $p < 0.01$) higher for males at 150 mg/kg/day compared to control group values at the recovery necropsy. These differences were considered to be a result of test article-related effects on final body weight. There were no other test article-related effects on organ weights.

-- MICROSCOPIC EXAMINATIONS

Test article-related microscopic changes were present in the liver of males at 150 mg/kg/day and in the kidneys of males at 75 and 150 mg/kg/day. There were no test article-related microscopic changes in females.

Incidence of Selected Histopathologic Findings in Males, Primary Necropsy (12 tissues examined per group): Data are presented in the following format: Finding / 0 mg/kg/day / 25 mg/kg/day / 75 mg/kg/day / 150 mg/kg/day

Liver:

- Centrilobular hypertrophy / 0 / 0 / 0 / 8+
- Minimal / 0 / 0 / 0 / 5+
- Mild / 0 / 0 / 0 / 3+

Kidney:

- Basophilic tubules / 4 / 3 / 3 / 9+
- Minimal / 4 / 3 / 2 / 8+
- Mild / 0 / 0 / 1 / 1+
- Hyaline droplets / 2 / 0 / 8+ / 12+
- Minimal / 2 / 0 / 5+ / 1+
- Mild / 0 / 0 / 3+ / 11+

The liver of 8/12 males in the 150 mg/kg/day group had minimal to mild centrilobular hepatocellular hypertrophy that ranged in severity from minimal to mild (grades 1-2, respectively, on a 1-4 scale). The lesions

were concentrated in the central region of the classical hepatic lobule and consisted of swollen hepatocytes.

Hyaline droplets, appearing as intracytoplasmic, brightly eosinophilic, partially refractile material, were present in proximal tubular epithelial cells of kidneys in 2/12, 0/12, 8/12 and 12/12 males in the control, 25, 75 and 150 mg/kg/day groups, respectively. Basophilic tubules, graded as minimal to mild, were present in the kidneys of 4/12, 3/12, 3/12 and 9/12 males in the same respective groups.

There were no test article-related changes in livers and kidneys examined from recovery males.

There were no other test article-related histologic changes. Remaining histologic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than administration of the test article. There was no test article-related alteration in the incidence, severity or histologic character of those incidental and spontaneous tissue alterations. Intracytoplasmic hyaline droplet formation in the renal proximal tubular cells of male rats is most often associated with accumulation of alpha 2 μ -globulin, which is specific to male rats. Renal tubular regeneration ("basophilic tubules") is commonly associated with alpha 2 μ -globulin nephropathy. Renal effects in male rats resulting from chemicals that cause alpha 2 μ -globulin accumulation are generally not expected to cause similar renal effects in humans (Hard, 1993). Hepatocellular centrilobular hypertrophy is considered a non-adverse adaptive response and is consistent with hepatic enzyme induction.

REPRODUCTIVE PERFORMANCE

No test article-related effects on mating or fertility were observed in males or females at any dosage level. Mating indices were 100.0%, 100.0%, 100.0% and 91.7% each in the control, 25, 75 and 150 mg/kg/day groups (males) and control, 10, 25 and 75 mg/kg/day groups (females), respectively. Fertility indices were 91.7%, 100.0%, 100.0% and 91.7%, and male copulation and female copulation indices were 91.7%, 100.0%, 100.0% and 100.0% in the same respective groups. The mean numbers of days between pairing and coitus in the test article-treated groups were similar to the control group value. None of these differences were statistically significant and none were attributed to the test article.

GESTATION LENGTH AND PARTURITION

Mean gestation lengths in the 10, 25 and 75 mg/kg/day groups were similar to those in the control group; differences were not statistically significant. No signs of dystocia were noted in these groups.

F1 LITTER DATA

-- POSTNATAL DAY (PND) 0 LITTER DATA AND POSTNATAL SURVIVAL

The mean number of pups born, live litter size and the percentage of males at birth in the 10, 25 and 75 mg/kg/day groups were similar to the control group values. Postnatal survival from birth to PND 4 in these groups was unaffected by parental test article administration.

-- GENERAL PHYSICAL CONDITION AND MORTALITIES

The numbers of F1 pups found dead and/or missing, as well as the general physical condition of all F1 pups in this study, were unaffected by parental

test article administration. Pups (litters) that were found dead numbered 6(4), 4(4), 6(4) and 1(1) in the control, 10, 25 and 75 mg/kg/day groups, respectively. One pup each in the control, 25 and 75 mg/kg/day groups was missing and presumed to have been cannibalized.

-- OFFSPRING BODY WEIGHTS

---- 75 mg/kg/day dose: Mean male and female pup body weights in the 75 mg/kg/day group (6.5 g and 6.0 g, respectively) on PND 1 were 11.0% and 10.4% lower, respectively, than control group values (7.3 g and 6.7 g, respectively); the values were below the minimum mean values in the WIL historical control data (6.7 g and 6.3 g, respectively), and differences from the control group were statistically significant ($p < 0.05$). These lower pup body weights at 75 mg/kg/day were considered test article-related. Mean male and female pup weights in this group (8.9 g and 8.2 g, respectively) on PND 4 remained lower than the minimum mean values in the WIL historical control data (9.0 g and 8.6 g respectively), but were no longer statistically significantly different from the concurrent control group values (9.9 g and 9.2 g, respectively). Mean pup body weight gains in the 75 mg/kg/day group during PND 1-4 were comparable to control group values; none of the differences were statistically significant.

---- 25 mg/kg/day and 10 mg/kg/day doses: Mean male and female pup body weights and body weight changes during PND 1-4 were unaffected by parental test article administration. No statistically significant differences from the control group were noted.

-- NECROPSIES OF PUPS FOUND DEAD

The numbers of pups (litters) found dead during PND 0-4 numbered 6(4), 4(4), 6(4) and 1(1) in the control, 10, 25 and 75 mg/kg/day groups, respectively. Renal papillae not developed and distended ureters were noted for a single fetus in the 25 mg/kg/day group. Milk was present in the stomach of 3(2) pups (litters) in the 25 mg/kg/day group.

-- SCHEDULED PUP NECROPSIES

No internal findings that could be attributed to parental test article administration were noted at the necropsy of pups euthanized on PND 4. A hemorrhagic ring was noted around the iris of 1 pup in the control group and 3 pups in the 25 mg/kg/day group. This finding did not occur in a dose-related manner; therefore, it was not attributed to parental test article administration. Renal papillae not fully developed (Woo and Hoar grade 1) were noted for 1(1) and 8(2) pups in the control and 25 mg/kg/day groups, respectively, and a renal papilla not developed or a distended ureter (Woo and Hoar grade 0) were noted for 2(2) pups in the 75 mg/kg/day group. These developmental variations are common in this strain of rat, and thus were not attributed to parental test article administration. No other internal findings were noted.

Source : Chevron Phillips Chemical Company LP, 2006. A Combined 28-Day Repeated Dose Oral Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of 3-Sulfolene in Rats, with Recovery - Audited Draft Report. Study performed by WIL Research Laboratories, LLC, Ashland, Ohio.

Test condition : TEST ANIMALS

CrL:CD(SD) rats from Charles River Laboratories. Animals were approximately 10 weeks old at the initiation of the study. Animal weights ranged from 331 to 369 g for males and 218 to 249 g for females on study day 0.

Animal Diet and Conditions: The basal diet used in the study was Certified

Rodent LabDiet 5002 (PMI Nutrition International, LLC). Reverse osmosis-purified drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the acclimation period (10 days) and during the study. All rats were housed throughout the acclimation period and during the study in an environmentally controlled room. Mean daily temperature ranged from 21.3 to 21.7 deg C and mean daily relative humidity ranged from 41.1 to 59.2% during the study. A 12-hour light/12-hour dark photoperiod was provided. Air handling units provided a minimum of 10 fresh air changes per hour.

VEHICLE

100% Mazola corn oil stored at room temperature (exp. dates: 7 Dec 2006, 29 Dec 2006, or 7 March 2007).

TEST SUBSTANCE

Dosing formulations prepared at test article concentrations ranging from 2.5 to 37.5 mg 3-sulfolene/mL were analyzed to confirm test article concentration and the results met the SOP acceptance criteria for test article concentration in suspension formulations, i.e, the analyzed concentrations were within 85% to 115% of the target concentrations, with the following exception. The 37.5 mg/mL formulation prepared on 30 November 2005 for administration to males in Group 5 (150 mg/kg/day) was 79.6% of the target concentration. That formulation was reanalyzed and the next formulation scheduled to be dispensed was resuspended, sampled and analyzed the following day (1 December 2005). The results met the SOP requirement for concentration acceptability for suspension formulations.

ORGANIZATION OF TEST GROUPS, DOSAGE LEVELS AND TREATMENT REGIMENS

The vehicle and test article formulations were administered orally by gavage once daily.

Males were dosed during the study days 0-27 (14 days prior to pairing through 1 day prior to scheduled euthanasia), for a total of 28 doses. At the end of the 28-day period, males assigned to the recovery groups remained on study for a 14-day recovery period without treatment.

Females were dosed during study days 0 through the day prior to euthanasia (14 days prior to pairing through lactation day 3) for a total of 40 to 44 doses. Females with no evidence of mating were dosed through the day prior to euthanasia to a total of 52 doses. Following 39 doses, females assigned to the recovery groups remained on study for a 14-day recovery period without treatment.

Dosage Volume: 4 mL/kg for all groups. Individual dosages were based on the most recent recorded body weights. All animals were dosed at approximately the same time each day.

Number of Animals Dosed:

- Vehicle: 18 animals/sex
- 25 mg/kg/day (males): 12 animals
- 10 mg/kg/day (females): 12 animals
- 75 mg/kg/day (males): 12 animals
- 25 mg/kg/day (females): 12 animals
- 150 mg/kg/day (males): 18 animals
- 75 mg/kg/day (females): 18 animals

At the end of the study, 6 animals/sex in the control and high-dose groups

remained on study for 14 days without treatment.

Dosage levels were selected based on the results of a 14-day pilot study.

The rats selected for the reproduction phase were paired for mating in the home cage of the male. Following positive evidence of mating, the males were housed in suspended wire-mesh cages until the scheduled necropsy, and the females were transferred to plastic maternity cages with nesting material (ground corncob bedding).

BREEDING PROCEDURES

The animals were paired on a 1:1 basis within each treatment group following 14 days of treatment for the males and females. A breeding record containing the male and female identification numbers and the start date of cohabitation was prepared. Each female was housed in the home cage of the male. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm following a vaginal lavage. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. If evidence of copulation was not detected after 14 days of pairing, any females that had not shown evidence of mating were placed in plastic maternity cages. For the purpose of calculating pre-coital intervals, rats paired over a 12-hour dark cycle were considered to have been paired for 1 day.

PARTURITION

All females were allowed to deliver naturally and rear their young to PND 4. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. On the day parturition was initiated (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery started.

PARAMETERS EVALUATED

-- All rats were observed twice daily (morning and afternoon) for morbidity and mortality. Detailed physical examinations were recorded weekly.

-- BODY WEIGHTS: Recorded weekly. Female body weights were recorded weekly until evidence of copulation was observed. Following copulation, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17, and 20 and on lactation days 1 and 4.

-- FOOD CONSUMPTION: Recorded on the corresponding weekly body weight days until pairing. Food intake not recorded during the mating period. Once evidence of mating was observed, female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Following mating, food consumption for females with no evidence of mating and for all males was measured on a weekly basis until the scheduled euthanasia.

-- FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

---- Home Cage Observations: Posture, biting, convulsions/tremors, palpebral (eyelid) closure, feces consistency.

---- Handling Observations: Ease of removal from cage, ease of handling animal in hand, lacrimation/chromodacryorrhea, salivation, piloerection, fur appearance, palpebral closure, respiratory rate/character, eye prominence,

mucous membranes/eye/skin color, red/crusty deposits, muscle tone.

---- Open Field Observations (Evaluated over a 2-minute observation period): Mobility, gait, rearing, arousal, convulsions/tremors, urination/defecation, grooming, gait score, bizarre/stereotypic behavior, backing, time to first step (seconds).

---- Sensory Observations: Approach response, touch response, startle response, tail pinch response, pupil response, eyeblink response, forelimb extension, hindlimb extension, air righting reflex, olfactory orientation.

---- Neuromuscular Observations: Hindlimb extensor strength, grip strength-hind and forelimb, hindlimb foot splay, rotarod performance.

---- Physiological Observations: Catalepsy, body weight, body temperature.

-- LOCOMOTOR ACTIVITY: Locomotor activity counts were recorded for 6 animals/sex/group following approximately 28 days of dose administration (males) and on lactation day 4 (females). Locomotor activity was measured automatically using the San Diego Instruments, Inc., Photobeam Activity System (San Diego Instruments, Inc., San Diego, California). Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills and ambulatory motor activity.

-- CLINICAL PATHOLOGY: Blood samples for clinical pathology evaluations (hematology and serum chemistry) were collected from 6 animals/sex/group at the scheduled necropsies. These animals were not fasted overnight prior to blood collection. Urine was collected from the 6 recovery phase rats/sex in the control and high-dose groups. The following parameters were evaluated:

---- Hematology: Total leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count (percent and absolute), differential leukocyte count (percent and absolute, neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell).

---- Serum Chemistry: Albumin, total protein, globulin (by calculation), albumin/globulin ratio (by calculation), total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium.

---- Urinalysis: Bilirubin, color, clarity, glucose, ketones, leukocytes, microscopy of sediment, occult blood, pH, protein, specific gravity, total volume.

-- MACROSCOPIC EXAMINATIONS

---- Unscheduled Death

---- Scheduled Euthanasia: All surviving adults were euthanized by carbon dioxide inhalation.

---- Necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera. At the time of necropsy, the following tissues and organs were placed in 10% neutral-buffered formalin: Adrenal glands, aorta, bone with marrow (sternabrae), bone marrow smear (not taken from animal found dead, not placed in formalin, examined only if warranted), brain (forebrain, midbrain, hindbrain), coagulating glands, eyes with optic nerve (placed in Davidson's

solution), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), heart, kidneys, exorbital lacrimal glands, liver (sections of 2 lobes), lungs (including bronchi, fixed by inflation with fixative), lymph node (mesenteric and mandibular), mammary gland (females only), ovaries and oviducts, pancreas, peripheral nerve (sciatic), pituitary gland, prostate gland, salivary gland (mandibular), seminal vesicles, skeletal muscle (rectur femoris), skin, spinal cord (cervical, thoracic and lumbar), spleen, testes with epididymides (fixed in Bouin's solution), thymus, thyroids, trachea, urinary bladder, uterus with vagina (uterus not taken from females found to be nongravid), all gross lesions.

---- Organ Weights: The following organs were weighted from all animals at the scheduled necropsies: Adrenal glands, brain, epididymides (weighed separately), heart, kidneys, liver, ovaries and oviducts, spleen, testes, thymus gland, thyroids with parathyroids. Absolute weights and organ to final body weight ratios were reported.

-- MICROSCOPIC EXAMINATIONS: Protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin, with the following exceptions. PAS staining was used for the testes and epididymides. The testes were fixed in Bouin's solution and embedded in paraffin. Microscopic examination was performed on all tissues listed above from all animals selected for the reproduction phase in the control and 150 mg/kg/day (males) or 75 mg/kg/day (females) groups at the scheduled necropsies, and from females that died or that failed to deliver. Based on the results of these evaluations, livers and kidneys from low- and mid-dosage group males, as well as from all recovery phase males, were also examined microscopically. Recovery phase females were not examined microscopically.

Because hyaline droplets were observed for male rats in the control, 75 and 150 mg/kg/day groups, immunohistochemical staining of the kidney tissue was performed for 5 male rats each in the control and 150 mg/kg/day groups in order to evaluate the presence of alpha-2u-globulin. The control rats selected included at least 1 animal observed microscopically with hyaline droplet formation. One tissue block from each male rat was processed. From each block, 2 sections of approximately 3-micron thickness were collected. One section was used as negative control, and the second section was incubated with the antibody. The evaluation of alpha-2u-globulin was performed via routine light microscopy.

EVALUATION OF F1 LITTERS

-- LITTER VIABILITY AND DEATHS: Each litter was examined daily for survival, and all deaths were recorded. A daily record of litter size was maintained. Intact offspring dying between birth and PND 4 were necropsied using a fresh dissection technique including the heart and major vessels (Stuckhardt and Poppe, 1984). Tissues were preserved in 10% neutral buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. The carcass of each pup was then discarded.

-- CLINICAL OBSERVATIONS: Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1 and 4. Any abnormalities in nursing behavior were recorded.

-- BODY WEIGHTS: Pups were individually weighed on PND 1 and 4. Mean pup weights were presented by sex for each litter and by dose group.

-- SEX DETERMINATION: Pups were individually sexed on PND 0 and 4.

-- SCHEDULED EUTHANASIA: On PND 4, all surviving F1 rats were euthanized by an intraperitoneal injection of sodium pentobarbital and necropsied with emphasis on developmental morphology.

STATISTICAL ANALYSIS

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation and the number of animals used to calculate the mean. In addition, percent change from control is presented for body weights, clinical pathology parameters and organ weights. Data obtained from nongravid females were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit.

Parental mating, fertility, conception and copulation indices were analyzed using the Chi square test with Yates' correction factor (Hollander and Wolfe, 1999). Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of corpora lutea and implantation sites, number of pups born, live litter size on PND 0, unaccounted-for sites, absolute and relative organ weights, clinical pathology values (excluding differential white cell counts other than lymphocytes and neutrophils), pre coital intervals and FOB data values were subjected to a parametric one way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-treated groups to the control group. FOB parameter that yield scalar or descriptive data in the test article-treated groups were compared to the control group using Fisher's Exact test (Steel and Torrie, 1980). Mean litter proportions (percent per litter) of males at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA (Kruskal and Wallis, 1952) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test article-treated groups to the control group.

For locomotor activity, total counts were analyzed by sex and session, with a repeated measure analysis of variance (RANOVA). Factors in the model included treatment group (TRT), time interval (TIME) and the interaction of time interval and treatment group (TRT*TIME). The SAS procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC) for first-order autoregressive homogeneous ([AR(1)]) and compound symmetric (CS) structures. The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dosage levels. The linear dose by time interaction (LinTrt*Time) was evaluated, and if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals by session only.

Nonmonotonic dose responses were evaluated whenever no significant linear trends were detected by TRT and/or TRT*TIME interaction was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted across the pooled time intervals of the entire

session. These nonmonotonic dose response comparisons were conducted at the 0.01 significance level.

All statistical analyses were conducted using SAS version 8.2 (SAS Institute, Inc., 1999-2001) software. Total count locomotor activity data were analyzed by BioSTAT Consultants, Inc., Portage, Michigan.

Ambulatory counts measured in the locomotor activity assessment were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup variance. If significant differences were indicated by the ANOVA, Dunnett's test (Dunnett, 1964) was used to compare the control and test article-treated groups.

Test substance Conclusion

- : 3-sulfolene (CAS# 77-79-2, 98.9% pure)
- : Based on the lack of test article-related reproductive effects at 25, 75 and 150 mg/kg/day for males and at 10, 25 and 75 mg/kg/day for females, 75 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for reproductive toxicity of 3-sulfolene when administered orally by gavage to CrI:CD(SD) rats. Based on the lower mean body weights, body weight gains and food consumption at 75 mg/kg/day (males and females) and 150 mg/kg/day (males), a dosage level of 25 mg/kg/day was considered to be the NOAEL for systemic toxicity. The NOAEL for neonatal toxicity was 25 mg/kg/day based on lower mean male and female pup weights on PND 1 and 4 in the 75 mg/kg/day group.

Reliability Flag
09.11.2006

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

(6) (7) (11) (20) (22) (27) (31) (32) (39)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT