

Robust Summary Document

N-oxydiethylenebenzothiazole-2-sulfenamide

CAS# 102-77-2

Molecular Formula: C11-H12-N2-O-S2
Molecular Weight: 252.3

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Off-white to tan solid
C. Purity: 95-99% Typical for Commercial Products

1.2 SYNONYMS Morpholine, 4-(2-benzothiazolylthio)-
N-oxydiethylenebenzothiazole-2-sulfenamide
Santocure® MBS
Santocure® MOR
Cure-Rite® OBTS
Delac® MOR
Vulkacit® MOZ
AMAX®
MOR
MBS
OBTS

1.3 IMPURITIES Morpholine (110-91-8) <0.4%
2-Mercaptobenzothiazole (149-30-4) <1%
Mercaptobenzothiazole Disulfide (120-78-5) <1%
Benzothiazole (95-16-9) <0.4%

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value: 82-88°C
Decomposition: No
Sublimation: No
Method: ASTM D-1519/FF83.9-1
GLP: Yes
Remarks: Capillary Melt Point determination
Reference: (1) ASTM D-1519/Flexsys Standard Methods of Analysis, 1983
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Value: 81.9-84.79°C
Decomposition: No
Sublimation: No
Method: Differential Scanning Calorimeter (DSC)
GLP: Yes
Remarks: Onset at 81.9°C. Final melt at 84.79°C
Reference: (2) Flexsys Analytical Research Report 2003.012, 2003
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

*2.2 BOILING POINT

Value: 216.1°C (Decomposition Temperature)
Pressure: 1 Atm
Decomposition: Yes
Method: Differential Scanning Calorimeter (DSC)
GLP: Yes
Remarks: Decomposition begins at 216.1°C, complete at 219.37°C
Conditions were 5°C/minute under nitrogen
Reference: (2) Flexsys Analytical Research Report 2003.012, 2003
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

†2.3 DENSITY (relative density)

Type: Density
Value: 1.4
Temperature: 20 °C
Method: FF97.8-1 Density of Solids
GLP: Yes
Remarks: Density of solids by displacement
Reference: (3) FF97.8-1 Flexsys Standard Methods of Analysis, 1997
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

*2.4 VAPOUR PRESSURE

Value: 0.000002013 hPa
Temperature: 25 °C
Method: Calculated – Antoine Method
MPBPWIN v1.40
GLP: No
Remarks: Calculated using MP = 82°C
Reference: (4) EPIWIN/MPBPWIN v1.40
Reliability: (2) Valid with restrictions – modelling data
Flag: Critical study for SIDS endpoint

Value: 0.0000023 hPa
Temperature: 20 °C
Method: Calculated
GLP: No
Remarks: None
Reference: (5) Bayer AG Data

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow: 3.49
Temperature: 25 °C
Method: Measured
HPLC Method for Pow
GLP: No
Remarks: 1% and .01% solutions in 100 ml n-Octanol added to 500 ml water.
Shaken for 48 hours, equilibration for several days.
Equilibration performed in the dark to preclude photodegradation.
Analysis via HPLC to determine Pow.
Pow = 3100 +/- 1400
Reference: (6) Monsanto ES-78-SS-20, 1978
Reliability: (2) Valid with restrictions – lack of method detail
Flag: Critical study for SIDS endpoint

*2.6 WATER SOLUBILITY

A. Solubility

Value: 0.039 mg/l (Degradation noted)
Temperature: 25 °C
Description: Of very low solubility
Method: HPLC of saturated water solution
GLP: No
Remarks: 1g of test chemical added to 500ml water. Stirred in the dark for several days. Analysis via HPLC. One or more degradation

products observed in the chromatogram. Results indicate the maximum amount of test compound in aqueous phase when a large excess is equilibrated with water.

Reference: (6) Monsanto ES-78-SS-20, 1978
Reliability: (2) Valid with restrictions – unstable compound under test conditions
Flag: Critical study for SIDS endpoint

2.7 FLASH POINT

Value: 177°C
Type: Cleveland Open Cup
Method: ASTM D 92-96
Reference: (7) Flexsys America Data
Reliability: (1) Valid without restrictions

Value: 188°C
Type: Open Cup
Method: DIN 51584
Reference: (5) Bayer AG data
Reliability: (1) Valid without restrictions

2.13 ADDITIONAL DATA

B. Other data – Henry's Law Constant

Results: 7.551E-008 atm-m³/mole
Remarks: Calculated at 25°C
Reference: (4) EPIWIN/HENRYWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type: Air
Indirect Photolysis:
Type of sensitizer: OH
Concentration of sensitizer: 156000 molecule/m³
Rate constant (radical): 0.000000001199482 cm³/molecule*sec
Degradation: 50% after 1.070 hours
Method: Calculated
AOP Program v1.89, 1999
GLP: No
Test substance: Other (calculated)
Reference: (4) AOPWin v1.89
Reliability: (2) Valid with restrictions – accepted calculation method
Flag: Critical study for SIDS endpoint

Type: Water
Light source: Sunlight
Spectrum of substance: 300 nm
Concentration of Substance: 1.006 mg/l
Temperature: 26 °C
Direct photolysis:
Half life: 1 hour
Degradation: 97 % after 4 hours
Method: Measured
Method similar to ASTM Draft Method No. 6, ASTM E35.24
Subcommittee, Aqueous Photolysis Task Group, 1980
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: Dark control recovery was 97% at 4 hours. Test substance values at 0, 1, 2, 3, and 4 hours were 100%, 45%, 15% and 3%, respectively, for light exposed conditions.
Reference: (8) Monsanto MO-92-9058/ES-80-SS-43, 1980
Reliability: (1) Meets National standards method (ASTM/AFNOR/DIN)
Flag: Critical study for SIDS endpoint

*3.1.2 STABILITY IN WATER

Type: Abiotic (hydrolysis)
Degradation: 24% at pH 7 at 20 °C after 25 hours
100% at pH 7 at 20°C after 7 days
Method: Phase I Hydrolysis Screening Study
H. Suffet, et. al., Hydrolysis Protocols – Effects of Water
On the Environmental Fate of Chemicals, 1980
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96%

Remarks: The experiment was run under dark conditions. Detection of hydrolysis products was performed by HPLC and GC/MS.
Hydrolysis products:
Benzothiazole (95-16-9), 64%
2-Mercaptobenzothiazole (149-30-4), 21%
Unknown, proposed comp. C11-H14-S2-N2-O2, 15%
Morpholine (110-91-8), % not determined

Reference: (9) Monsanto AB-84-131, Analytical Bio-Chemistry Labs, 1984

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

3.2 MONITORING DATA (ENVIRONMENTAL)

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

*3.3.1 TRANSPORT

Type: Volatility

Media: Water

Method: Estimation Method, 1990

Results: Volatilization half-life from model river: 1.71e+008 hours
Volatilization half-life from model lake: 1.865e+009 hours
Volatilization Constant from water: 5.44e-012 atm-m³/mole

Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec.
Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.

Reference: (10) Handbook of Chemical Property Estimation Methods, 1990

Reliability: (2) Valid with restrictions – modelling data

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota-sediment-soil-water

Method: Fugacity level III
EPIWIN v3.10

Results:	<u>Mass Amount (%)</u>	<u>Half-life (hrs)</u>	<u>Emissions (kg/hr)</u>
Air	7.5e-005	2.14	1000
Water	20.3	900.00	1000
Soil	78.8	900.00	1000
Sediment	0.944	3.6e+003	0

Persistence time estimated at 1.03e+003 Hours

Remarks: Calculations based on user input values of water solubility of 24.01 mg/l, Log Kow of 3.49, and melting point of 82°C

Reference: (4) EPISUITE/EPIWIN v3.10

Reliability: (2) Valid with restrictions – modelling data

Flag: Critical study for SIDS endpoint

***3.5 BIODEGRADATION**

Type: Aerobic
Inoculum: Predominately domestic sewage
Concentration of the chemical: 100 mg/l related to COD (Chemical Oxygen Demand)
Degradation: 0% after 28 days
Results: Under test condition no biodegradation observed
Method: Directive 84/449/EEC, C.7, Biotic Degradation – Modified MITI
GLP: No
Test substance: As prescribed by 1,1-1.4, purity: >93%
Reference: (5) Bayer AG data
Reliability: (1) Valid without restriction (Meets national standards method)
Flag: Critical study for SIDS endpoint

3.6 BOD5, COD or BOD5/COD Ratio

ThOD: 1880 mg/g
Reference: (5) Bayer AG data

3.7 BIOACCUMULATION

Species: Other
BCF: 100 (+/-30)
Method: Calculation, Neely, et. al., 1974
GLP: No data
Remarks: Calculation from measured octanol/water partition coefficient
Pow = 3100 (+/- 1400)
Reference: (6) Monsanto ES-78-SS-20, 1978
Reliability: (2) Valid with restrictions – acceptable calculation method

Species: Other
BCF: 97.12
Method: BCFWIN v2.14
GLP: No
Remarks: Calculation using measured Log Pow = 3.49.
Excellent agreement with calculated BCF of 100 (above)
Reference: (4) EPIWIN/BCFWIN v2.14
Reliability: (2) Valid with restrictions – modelling data

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test:	Static Closed-system
Species:	<i>Lepomis macrochirus</i> (fish, fresh water)
Exposure period:	96 hours
Results:	LC ₅₀ (96h) = 11.5 mg/l
Analytical monitoring:	Yes
Method:	Other: Standard Methods for the Examination of Water and Wastewater, 13 th ed., American Public Health Association, 1971
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >93%
Remarks:	Test fish (1-3" in length) were obtained from a state licensed commercial fish hatchery and were retained for at least 10 days before use. The dilution water was natural pond water with hardness expressed as 44 ppm CaCO ₃ . A saturated aqueous extract (SAE) was made by allowing the test material to sit in distilled water after vigorous shaking for at least 24 hours. The SAE was filtered and then tested. All tests were conducted at room temperature (22°C), pH 6.5-8.5, and all dissolved oxygen levels remained above 4 mg/l at all times during the tests.
Reference:	(11) Uniroyal Chemical Unpublished Report, 1972
Reliability:	(1) Valid without restriction – meets National standards method (AFNOR/DIN)
Flag:	Critical study for SIDS endpoint
Type of test:	Flow-through Open-system
Species:	<i>Oncorhynchus mykiss</i> (Rainbow Trout)
Exposure period:	144 hours
Results:	LC ₅₀ (24h) = 0.46 mg/l LC ₅₀ (96h) = 0.31 mg/l LC ₅₀ (144h) = 0.31 mg/l
Analytical monitoring:	Yes
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 96%.
Remarks:	The test was performed under continuous flow conditions using a Mount-Brungs diluter. It was conducted in 19-liter glass aquaria, with 10 fish/replicate. Flow rate was set to provide 4 tank volumes/day. Juvenile trout, obtained from Mt.Lassen Trout Farm, were maintained at SRI for at least two weeks prior to testing. Average fish length was 6.65 cm, and weight was 2.48 g. Fish were fed a daily ration (5% of body weight) of frozen brine shrimp. The stock solution of the test compound was prepared by mixing the test compound in acetone at a concentration of 40g/l. The stock solution was metered into the diluter with a syringe

pump set at 3 ml/min. The test solution was freshly made every 48 hours. A separate bottle was used to deliver a dilute acetone solution to maintain the concentration in the solvent control tanks at 150 ul/l. Nominal test concentrations were 0.00, 0.12, 0.25, 0.50, 1.00, 2.00 mg/l plus the solvent control. The test was terminated after 6 days of exposure, as no deaths had occurred for two consecutive days. Dissolved oxygen (DO), pH, temperature and chemical concentrations were measured routinely, alternating between the replicates. DO ranged from 8.6 to 11.8 mg/l, pH from 7.7 to 8.9, temperature between 12.0 and 13.0°C. Hardness, as CaCO₃, was 27 mg/l, alkalinity was 24 mg/l, and acidity was <5.0 mg/l. Test chemical concentrations were determined by an internal standard HPLC method. Measured concentrations of the test chemical were less than the nominal concentrations, indicating that degradation was taking place. The test substance exhibited a high acute toxicity to rainbow trout under the test conditions.

Reference: (12) Monsanto SR-80-66, SRI International, 1981
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Type of test: Static
Closed-system

Species: *Pimephales promelas* (Fathead Minnow)

Exposure period: 96 hours

Results: LC₅₀ (24h) = 4.0 mg/l
LC₅₀ (48h) = 4.0 mg/l
LC₅₀ (96h) = 3.5 mg/l
NOEC = 1.0 mg/l
LOEC = Not Determined

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972).

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 96%

Remarks: Test fish were obtained from Fattig Fish Hatchery in Brady, Nebraska. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. Test fish had a mean weight of 0.25 g and a mean standard length of 25.8 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of laboratory well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm, hardness (CaCO₃) of 255 ppm and pH 8.2. The test vessels were kept in a water bath at 22°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde acetone was used to prepare the test solutions and as the solvent control. A 48-hour range-finding study preceded the definitive test. Test concentrations were 0, 1.0, 1.8, 3.2, 5.6 or 10.0 mg/l for the test compound. Fish were placed in

the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values and pH ranges were monitored during the testing and remained within acceptable limits of 40-100% saturation for dissolved oxygen and pH value consistent with control. The ammonia concentration was below the toxic limit. Water hardness (CaCO₃) was 255 ppm. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. These values were obtained by employing the statistical methods described by Litchfield and Wilcoxon (A Simplified Method for Evaluating Dose-Effect Experiments, 1949) or Stephan (Methods for Calculating an LC₅₀, 1977).

Reference: (13) Monsanto AB-80-0240, Analytical Bio-Chemistry Labs, 1980
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Type of test: Static
Closed-system
Species: *Lepomis macrochirus* (Bluegill Sunfish)
Exposure period: 96 hours
Results: LC₅₀ (24h) = >7.5 mg/l
LC₅₀ (48h) = 6.0 mg/l
LC₅₀ (96h) = 4.4 mg/l
NOEC = 3.7 mg/l
LOEC = 4.2 mg/l

Analytical monitoring: No
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972).

GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96%.

Remarks: The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations ranged from 3.7-10.0 mg/l for the test compound. Ten bluegill, standard length 3.8 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 22°C. Dissolved oxygen content ranged from 8.8 mg/l (100% of saturation) at the beginning of the test, to 0.7 mg/l (8% of saturation) at the end of the exposure period. Beginning pH was 7.5; ending pH was 6.7. Water hardness (CaCO₃) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC₅₀ values and the 95% confidence intervals were calculated from the regression equation.

Reference: (14) Monsanto BN-76-172, EG&G Bionomics, 1976
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Type of test: Static
Closed-system

Species: *Oncorhynchus mykiss* (Rainbow Trout)

Exposure period: 96 hours

Results: LC₅₀ (24h) = 5.3 mg/l
LC₅₀ (48h) = 1.4 mg/l
LC₅₀ (96h) = 1.3 mg/l
NOEC = 1.0 mg/l
LOEC = 1.4 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972)

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: 96%.

Remarks: The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations ranged from 1.0 to 10.0 mg/l for the test compound. Ten rainbow trout, standard length 2.7 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 12°C. Dissolved oxygen content ranged from 9.8 mg/l (92% of saturation) at the beginning of the test, to 2.5 mg/l (23% of saturation) at the end of the exposure period. Beginning pH was 7.7; ending pH was 6.8. Water hardness (CaCO₃) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC₅₀ values and the 95% confidence intervals were calculated from the regression equation.

Reference: (14) Monsanto BN-76-172, EG&G Bionomics, 1976

Reliability: (1) Valid without restriction

Type of test: Static
Closed-system

Species: *Brachydanio rerio* (fish, fresh water)

Exposure period: 96 hours

Results: LC₀ (96h) = 1 mg/l
LC₁₀₀ (96h) = 5 mg/l

Analytical monitoring: No

Method: No data, 1989

GLP: No

Test substance: As prescribed by 1.1-1.4, purity: no data

Remarks: The test substance was given in water and stirred for 24 hours using a magnetic stirrer. At all test concentrations, undissolved particles remained in the medium.

Reference: (5) Bayer AG data

Type of test: Static
Species: *Oncorhynchus kisutch* (fish, fresh water, marine)
Exposure period: 24 hours
Results: LC_{50} (24h) = >10 mg/l
Analytical monitoring: No
Method: No data
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: The compound was tested at 10 mg/l. Length of test fish was 5-10 cm.
Loss of equilibrium occurred in 7-10 hours.
Reference: (15) MacPhee and Ruelle, U. of Idaho, 1969
Reliability: (4) Not assignable – data from a secondary literature source

Type of test: Static
Species: *Oncorhynchus tshawytscha* (fish, fresh water, marine)
Exposure period: 24 hours
Results: LC_{50} (24h) = >10 mg/l
Analytical monitoring: No
Method: No data
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: The compound was tested at 10 mg/l. Length of test fish was 5-10 cm
Reference: (15) MacPhee and Ruelle, U. of Idaho, 1969
Reliability: (4) Not assignable – data from a secondary literature source

Type of test: Static, closed-system
Species: *Ptychocheilus oregonensis* (fish, fresh water)
Exposure period: 24 hours
Results: LC_{50} (24h) = >10 mg/l
Analytical monitoring: No
Method: No data
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: The compound was tested at 10 mg/l. Length of test fish was 5-10 cm
Reference: (15) MacPhee and Ruelle, U. of Idaho, 1969
Reliability: (4) Not assignable – data from a secondary literature source

Type of test: Static
Species: *Brachydanio rerio* (fish, fresh water)
Exposure period: 96 hours
Results: LC_0 at dilution 1:8
 LC_{100} at dilution 1:2
Analytical monitoring: No
Method: No data, 1988
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: The test compound was eluted in the concentration of 1g/l for

two hours, filtered, and then the filtrate was tested. DOC of the filtrate was 11 mg/l.
Reference: (5) Bayer AG data

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. *Daphnia*

Type of test: Static
Closed-system

Species: *Daphnia magna* (crustacean)

Exposure period: 48 hours

Results: EC₅₀ (24h) = 6.8 mg/l
EC₅₀ (48h) = 4.0 mg/l
NOEC = 1.0 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975

GLP: No

Test substance: As prescribed by 1.1-1.4, purity: 96%.

Remarks: The *Daphnia magna* used in the test were cultured at the ABC facilities. The adult *Daphnia* were fed the algae *Selenastrum capricornutum* at least every three days prior to testing and supplemented with a suspension of trout chow. The bioassay was conducted in 500-ml glass beakers containing 250 ml of ABC well water. Water quality parameters were DO = 8.7 mg/l, pH = 7.7, hardness = 220 mg/l, and alkalinity = 210 mg/l. Vessels were kept at 19°C in a temperature- controlled area. Lighting was maintained at 50-70 foot-candles on a 16-hour daylight photoperiod. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control. Ten daphnia (first instar less than 18 hours old) were selected for each of the five test concentrations (1.0 – 10.0 mg/l) in duplicate. *Daphnia* in all concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen levels and pH were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. DO was 7.8 mg/l and pH was 8.0 at the end of the testing. LC50 and 95% CI were calculated employing the technique of Litchfield and Wilcoxon.

Reference: (16) Monsanto AB-78-355, Analytical Bio-Chemistry Labs, 1978

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Type of test: Static
Closed-system

Species: *Paratanytareum parthenogenetica* (Midge)

Exposure period: 48 hours

Results: EC₅₀ (24h) = 8.6 mg/l
 EC₅₀ (48h) = 5.3 mg/l
 NOEC = 1.8 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972), and Methods of Conducting Acute Toxicity Tests with Midge (*Paratanytarsus parthenogenetica*) (1980)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 99%

Remarks: Test midge for this study were cultured at the ABC facilities. The adult midge were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The test was carried out using 3rd and 4th instar larvae, 8-10 days old. The static bioassay was conducted in 250-ml glass beakers containing 200-ml of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.2 mg/l, hardness (CaCO₃) of 255 ppm and pH 7.8. The test vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range finding experiment preceded the definitive bioassay. Nanograde Acetone was used to prepare the test solutions, which ranged from 1.0 to 18 mg/l, and as the solvent control. Tests were run in duplicate. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen content ranged from 9.2 to 8.7 mg/l and pH ranged from 8.3 to 7.8 during the testing. Water quality parameters of temperature, dissolved oxygen content and pH were measured at the termination of the test and were within acceptable limits. The LC50 values were calculated via a computerized program performing the following statistical tests: binomial, moving average and probit tests.

Reference: (17) Monsanto AB-81-357, Analytical Bio-Chemistry Labs, 1981

Reliability: (1) Valid without restriction

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Endpoint: Biomass and Growth rate

Exposure period: 96 hours

Results: Chlorophyll A. EC₅₀ (96h) = 2 mg/l
 Cell Count EC₅₀ (96h) = 2 mg/l
 NOEC = 0.6 mg/l
 LOEC = Not Determined

Analytical monitoring: No

Method: US EPA Phytotoxicity Method - Algal Assay Procedure: Bottle Test (1971)
 Closed-system

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: 96%

Remarks: The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 2.0 x 10⁴ cells/ml. Cultures were incubated at 24°C under approximately 4,000 lux illumination. Triplicate cultures were employed

for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 96-hr range-finding study. These concentrations were 0, 0.6, 1, 3, 6 or 10 mg/ml. Test concentrations were dissolved in reagent-grade acetone. The solvent control was 0.05 ml acetone in water. There were no water quality measurements reported in this study. The toxicity of the test compound appeared to reach a maximum after 28 hours of exposure. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of *in vivo* chlorophyll A or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression.

Reference: (18) Monsanto BN-73-363, EG&G Bionomics 1973
Reliability: (2) Valid with restrictions – no water quality data
Flag: Critical study for SIDS endpoint

***4.4 TOXICITY TO MICROORGANISMS, e.g. Bacteria**

Type: Aquatic
Species: Activated Sludge
Exposure period: 3 hours
Unit: ug/ml
Analytical monitoring: No
EC50: >10,000 ug/ml
Method: ISO 8192, Test for Inhibition of Oxygen Consumption by Activated Sludge, 1988
Test substance: As prescribed by 1.1-1.4, Purity not stated
GLP: No
Remarks: Direct weight
Reference: (19) Bayer AG data
Reliability: (1) Valid without restriction – meets National standard method

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type: LD₅₀
Species/strain: Rat, Sprague-Dawley
Sex: Male and female
Number of animals: 16
Vehicle: Corn oil
Value: 12,560 mg/kg bw
Method: Other: Industrial Bio-Test laboratories, Inc. Protocol, 1974
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: 90-94%
Remarks: Initial screen was conducted to determine general level of toxicity. Two rats/sex/dose were administered the test material by gavage. The rats were then housed individually and observed for 14 days. Initial and final body weights, mortalities and reactions were recorded. A necropsy examination was conducted on all animals. The acute oral median lethal dose was calculated using the techniques of Weil, CS (1952), Thompson, WR (1947), and Thompson, WR and Weil, CS (1952)
Results: Adverse reactions observed at:
4556 mg/kg: hyporeactivity and ruffed fur
6834 mg/kg: as above, plus salivation and labored breathing
10250 mg/kg: as above, plus muscular weakness, prostration and diuresis.

<u>Dose ug/kg</u>	<u>Mortality rate</u>	<u>% Mortality</u>
4566	0 of 4	0
6834	0 of 4	0
10205	1 of 4	25
15380	3 of 4	75

Reference: (20) BF Goodrich AN-3546, Industrial Bio-Test Labs, 1974
Reliability: (2) Valid with restrictions – meets generally accepted scientific standards, well-documented, and acceptable for assessment
Flag: Critical study for SIDS endpoint

Type: LD₅₀
Species/strain: Rat, Wistar
Sex: Male and female
Number of animals: 20
Vehicle: Propylene Glycol
Value: >10,000 mg/kg bw
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: not stated
Method: The test material was given as a 33% (w/v) suspension in propylene glycol to groups of 10 male and 10 female rats in a single dose of 30 ml/kg bw [10 g test substance/kg bw]. The animals received feed and water *ad libitum* during the fourteen

day observation period. The rats were observed daily for signs of intoxication and mortality. All animals were necropsied. No deaths occurred during the observation period. Microscopic examination did not reveal any treatment-related alterations.

Reference: (21) de Groot, A.P., CIVO-TNO, 1976
Reliability: (2) Valid with restrictions – meets generally accepted scientific standards, well-documented and acceptable for assessment
Flag: Critical study for SIDS endpoint

Type: LD₅₀
Species/strain: Rats, Sprague-Dawley Albino
Value: >7940 mg/kg bw
Sex: Male and female
Number of Animals: 15
Vehicle: Corn Oil
Doses: 5010, 6310, or 7940 mg/kg bw
Method: Single Oral Dose Protocol, Younger Labs, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96 %
Remarks: The test material was administered to three groups of male and female rats (5 animals/dose level) as a 25.0% suspension in corn oil. Male rats had initial average body weights of 210-235 grams; females had initial average body weights of 215-220 grams. Clinical signs of toxicity included reduced appetite and activity (one to three days in survivors), followed by increasing weakness, collapse, and death. There were no deaths in males at any dose level, and only one in females on Day 2 at the highest dose level. Surviving animals were sacrificed on Day 7. Gross autopsy findings on the decedent were hemorrhagic lungs and liver, and acute gastrointestinal inflammation. Findings on survivors were that all viscera appeared normal.

<u>Dose mg/kg</u>	<u>Mortalities-Male</u>	<u>Mortalities-Female</u>	<u>Combined</u>
5010	0/2	0/3	0/5
6310	0/3	0/2	0/5
7940	0/2	1/3	1/5

Reference: (22) Monsanto Y-73-194, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

Type: LC₅₀
Species/strain: Rat
Sex: Male
Number of animals: 10
Vehicle: None - undiluted test article
Exposure time: 1 hour
Value: >151 mg/l
Method: Other, technique specified in Federal Register, 08/12/1961
GLP: No

Test substance: As prescribed by 1.1-1.4, purity: Not noted
Remarks: The test substance was ground in a blender and filtered through a 40-mesh screen to produce a fine dust. During the one-hour exposure, animals generally exhibited excessive preening and masticatory movements, excessive salivation, and occasional periods of excited activity. Two rats died on the 4th or 10th day post-exposure. No other mortalities occurred. Signs of toxicity prior to mortalities were depression, depressed righting and placement reflexes, ataxia (1 animal, 10th day post-exposure), and diarrhea stains of 2 days duration (1 animal – 10th day post-exposure). Surviving rats exhibited normal appearance and behavior throughout the 14-day observation period.
Reference: (23) BF Goodrich T-99, Hill Top Research, 1969
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Type: LC₅₀
Species/strain: Rat
Sex: Male and female
Number of animals: 20
Vehicle: None – undiluted powder
Exposure time: 4 hours
Value: >0.09 mg/l
Method: Other – Industrial Bio-Test Laboratory Protocol, 1974
GLP: No
Test substance: As prescribed by 1.1-1.4
Method: Ten albino rats were exposed to the dust of the test material for four hours in a 70-liter chamber. The concentration was determined by sampling the test atmosphere in the animals' breathing zone and collection on a glass fiber filter. The average filter concentration was 0.09 mg/l of air. The 10 exposed animals and 10 untreated control animals were observed for 14 days.
Results: There were no deaths during exposure or the observation period. All rats in both treated and control groups gained weight during the study. There were no gross pathologic findings attributable to inhalation of the test material. Gross pathologic changes in test and untreated control animals were essentially the same.
Reference: (24) IBT #663-04730
Reliability: (2) Valid with restrictions- Meets generally accepted scientific standards, well-documented and acceptable for assessment

5.1.3 ACUTE DERMAL TOXICITY

Type: LD₅₀
Species/strain: Rabbits, New Zealand Albino
Value: >5010 mg/kg bw
Sex: Male and female
Number of Animals: 4

Vehicle: Corn Oil
 Doses: 3160, 5010, or 7940 mg/kg bw
 Exposure Time: 24 Hours
 Method: Other – Younger Laboratories Protocol, 1973
 Test substance: As prescribed by 1.1-1.4, purity 96%
 Method: The test substance, as a 40.0% suspension in corn oil, was applied to the shaved skin of three groups of male and female rabbits for 24 hours as single dermal application at dose levels of 3160, 5010 or 7940 mg/kg/body weight. Body weights of males were 2.3-2.4 kg, and females, 1.8-2.3 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter until sacrifice.

Results: Clinical signs of toxicity included reduced appetite and activity for one to three days. There were no mortalities. All animals were sacrificed on Day 14.

<u>Dose mg/kg</u>	<u>Mortalities-Male</u>	<u>Mortalities-Female</u>	<u>Combined</u>
3160	----	0/1	0/1
5010	0/1	---	0/1
7940	0/1	0/1	0/2

Reference: (22) Monsanto Y-73-194, Younger Laboratories, 1973
 Reliability: (2) Valid with restrictions – age of study, lack of method detail
 Flag: Critical study for SIDS endpoint

Type: LD₅₀
 Species/strain: Rabbit, New Zealand White
 Sex: Male and female
 Number of animals: 4
 Vehicle: None
 Value: >3000 mg/kg bw
 Method: Other: Industrial Bio-Test Laboratories Protocol, 1974
 GLP: No
 Test substance: As prescribed by 1.1-1.4
 Method: Young adult New Zealand albino rabbits were acclimated and examined prior to testing. Twenty-four hours prior to testing, the backs of the animals (approximately 30% total body surface) were shaved free of hair. The test material was applied as received to abraded, pre-moistened skin. The test material was applied at the highest reasonable dosage and the test site was covered with plastic sheeting. After 24 hours, the plastic sheeting and the test material were removed. The animals were examined for local skin reactions, behavioral abnormalities and mortality for 14 days. A necropsy was conducted on all animals.

Results: No animals died during the study.

Reference: (20) BF Goodrich AN-3546, Industrial Bio-Test Labs, 1974
 Reliability: (2) Valid with restrictions- Meets generally accepted scientific standards, well-documented and acceptable for assessment

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain: Rabbits
Concentration: 0.5 g undiluted
PDII: 0.8
Results: Slightly Irritating
Classification: Not a Primary Skin Irritant
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed by 1.1-1.4
Results: A dose of 0.5g of the undiluted test article was applied to the intact and abraded skin of albino rabbits and occluded for 24 hours.

<u>Sample tested</u>	<u>Primary Irritation Score</u>
OBTS 1-A	0.1
OBTS 1-B	0.2
OBTS 1-C	0.1
OBTS 1-D	0.8

Reference: (20) BF Goodrich AN-3546, Industrial Bio-Test Labs, 1974
Reliability: (2) Valid with restrictions- Meets generally accepted scientific standards, well-documented and acceptable for assessment

Species/Strain: Rabbits, New Zealand Albino
Concentration: 0.5 g undiluted
Number of animals: 6
PDII: 0.6
Results: Slightly Irritating
Classification: Not a Primary Skin Irritant
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: 0.5 grams of the test substance, as a finely ground powder moistened with water, was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours.

Reference: (25) Monsanto Y-73-191 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.2 EYE IRRITATION/CORROSION

Species/strain: Rabbits, New Zealand Albino
Concentration: 100 mg undiluted
Exposure time: 24 hours
Number of animals: 6

Results: Slightly Irritating
 Classification: Irritating
 Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
 GLP: No data
 Test substance: As prescribed in 1.1-1.4, purity: 96%
 Remarks: 100 mg of the test substance, as a finely ground powder, was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris, and conjunctivae were examined immediately after treatment, and then at intervals of 10 minutes, 1 hour, and then at 24, 48, 72, and 168 hours. The Draize Method was used for scoring eye irritation.

<u>Exposure time</u>	<u>Mean score (X/110)</u>
24 hours	12.0
48 hours	5.0
72 hours	1.0
168 hours	0.0

Reference: (25) Monsanto Y-73-191 Younger Laboratories, 1973
 Reliability: (2) Valid with restrictions – age of study, lack of method detail

Species/strain: Rabbits, New Zealand Albino
 Concentration: 100 mg undiluted
 Exposure time: 24
 Number of animals: 24
 Results: Moderately Irritating
 Classification: Irritating
 Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
 GLP: No data
 Test substance: As prescribed in 1.1-1.4, purity: not noted
 Remarks: Not rinsed
 Reference: (20) BF Goodrich AN-3546, Industrial Bio-Test Labs, 1974
 Reliability: (2) Valid with restrictions- Meets generally accepted scientific standards, well-documented and acceptable for assessment

***5.3 SENSITIZATION**

Type: Patch test
 Species: Human
 Number of animals: 49
 Dose: 75% active
 Vehicle: Petrolatum
 Result: Positive, 24/49
 Classification: Sensitizer
 Method: Shelanski & Shelanski Repeated Insult Patch Test, 1953
 Test substance: As prescribed by 1.1-1.4, purity 96%
 Remarks: The test substance, as a 75% preparation in petrolatum, was applied to the skin of 49 human volunteers. 24 of the 49 exhibited a sensitization reaction. No evidence of primary irritation observed.

Reference: (26) Monsanto SH-82-8, Product Investigations, Inc. 1982
 Reliability: (1) Valid without restriction

Type: Patch test
Species: Human
Number of animals: 30 and 21
Dose: 10% w/w or 1% w/w
Vehicle: Petrolatum
Result: Positive (23/30 @ 10%, 3/21 @ 1%)
Classification: Sensitizer
Method: Shelanski & Shelanski Insult Patch Test, 1953
Test substance: As prescribed by 1.1-1.4, purity 96%
Remarks: Induction phase was 4 days/week for three weeks, followed by a one week rest period. Challenge phase was 4 days/week on a naïve site for two consecutive weeks.
Reference: (27) Monsanto SH-86-255, Product Investigations, Inc. 1986
Reliability: (1) Valid without restriction

Type: Patch test
Species: Human
Number of animals: 20
Dose: 10% w/w
Vehicle: Petrolatum
Result: Negative
Classification: Not sensitizing (1/20)
Method: Shelanski & Shelanski Repeated Insult Patch Test, 1953
Test substance: Other TS: recrystallized, purity = 99%
Remarks: The test substance, highly purified via recrystallization, was tested for potential to induce a sensitization response. Induction phase was 4 days/week for three weeks, followed by a one week rest period. Challenge phase was 4 days/week on a naïve site for two consecutive weeks. Only one volunteer reacted to the purified material.
Reference: (27) Monsanto SH-86-255, Product Investigations, Inc. 1986
Reliability: (1) Valid without restriction

Type: *In vitro* Lymphocyte Transformation Assay
Species: Human
Result: Positive for oxidized impurities
Classification: Sensitizer
Method: Nowell, P.C., 1960
Test substance: As prescribed by 1.1-1.4, purity 96%
Other TS: 2-mercaptobenzothiazole
Other TS: Morpholine
Other TS: Santocure MOR sulfoxide (oxidized impurity)
Other TS: Santocure MOR sulphone (oxidized impurity)
Remarks: The test substance, along with four known impurities in the commercial-grade product, was tested on human lymphocytes obtained from the blood of seven previously sensitized volunteers (two males and five females who gave 4+ or greater reactions in the Shelanski Repeat Insult Patch Test) to see if DNA synthesis/stimulation would result. The test showed that the oxidized impurities are primarily responsible for the sensitization response.
Reference: (28) Monsanto ML-87-68, 1987

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Charles River CD
Sex: Male/Female
Number of animals: 50
Route of Administration: Oral feed
Exposure period: 4 weeks
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 100, 200, 500, or 1000 mg/kg bw
Control group: Yes
Concurrent no treatment
NOEL: 200 mg/kg
LOEL: 500 mg/kg
Method: Other – Hazleton Laboratories Europe Protocol, 1979
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity 97.3%
Method: The purpose of this range-finding study was to determine an appropriate dosage for a teratology study, to determine the stability of the test article on rat feed, and to determine the palatability of the test article/rat feed mixtures. Four groups of rats (5/sex/dose level) were fed diets containing the test substance for four consecutive weeks. A similar group of five males and five females served as controls, receiving only the basic powdered diet. The diet samples were analyzed via HPLC to determine actual dose levels and stability of the test material on the rat feed. Animals were observed twice daily during the first week of the study for signs of toxicity, and then at daily intervals thereafter. Body weight, food and water consumption were recorded weekly. No blood or urine analyses were conducted. All animals were subjected to a necropsy. Major organs and tissues were examined for gross lesions. Organ weights were recorded for adrenals, brain, heart, liver, spleen, kidneys, gonads, lungs and thyroid.

Results: All animals survived until terminal sacrifice. There were no changes in the animals' condition that could be related to treatment. Male rats at the two highest dose levels gained weight more slowly than controls from the first week of treatment. By the end of the study, the weight difference was approximately 12%. The livers of several high-dose males were found to be enlarged. Mean liver weights were also higher in this group. The mean relative kidney weight was higher in high-dose males than those of control animals.

Reference: (29) Monsanto HL-79-031, Hazleton Laboratories, 1979
Reliability: (2) Valid with restrictions- Meets generally accepted scientific standards, well-documented and acceptable for assessment
Flag: Critical study for SIDS endpoint

Species/strain: Rabbits, New Zealand White
Sex: Male/Female
Number of animals: 80
Route of Administration: Dermal
Exposure period: 21 days [1x/day for 6 hours, 5 days/week]

Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 125, 500, or 2000 mg/kg bw
Control group: Yes
Concurrent vehicle (0.9% physiological saline)
NOEL: 2000 mg/kg
LOEL: Not determined
Method: Other: International Research and Development Corporation Protocol, 1979
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.2%
Method: The test substance was administered to three groups of 10 male and 10 female rabbits (young adults, 2.0-3.5 kg bw), half with intact skin and half with abraded skin, five days/week for three consecutive weeks. An identical control group was treated with saline. Criteria evaluated for treatment effect included mortality, pharmacotoxic signs, body weights, dermal irritation, hematological and clinical biochemical determinations, organ weights, and macroscopic and selected microscopic evaluation of tissues. Rabbits were observed twice daily for mortality, once daily for pharmacotoxic signs and evidence of dermal irritation. Body weights were obtained and recorded during the pretest period and 1x/week thereafter. Once during the pretest period and on Day 21 of the study, laboratory tests were conducted on five rabbits/sex/group.

Results: One death (not considered to be treatment-related) occurred in each of the three test article groups, and one control animal was sacrificed *in extremis*. All remaining animals survived until terminal sacrifice. No test article-related signs were noted for any animal in the test groups during the study period. A number of incidental and spontaneous pharmacotoxic signs were noted for a few rabbits in all groups (nasal discharge, eye discharge, soft stool, possible anorexia). No statistically significant differences were seen in group mean body weights. A few rabbits in the test groups exhibited very slight to slight erythema, edema and desquamation. In the control group, a few rabbits exhibited very slight desquamation and red raised areas on the shaven backs. Although some differences were seen between control and treated rabbits in the hematological studies (slight decreases in hematocrit, hemoglobin and total erythrocytes in high-dose females, slightly lower LDH and higher cholesterol values in high-dose males), these differences were not considered to be related to treatment with the test article. Both the control and groups, and the males and females presented similar changes. The main lesions on the skin from the application site of control and experimental animals were hyperkeratosis and infiltration of inflammatory cells in the dermis. Body weights (Week 3), hematological, biochemical and urinalysis parameters (Day 20), and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-Test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge the significance of differences.

Reference: (30) Monsanto IR-79-264, International Research & Development Corp., 1981

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

Species/strain: Rat, Sprague-Dawley
Sex: Male/Female
Number of animals: 40
Route of Administration: Inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: None
Dose: 0, 4.4, 9.8, or 10.2 mg/m³
Control group: Yes
Concurrent no treatment
NOEL: 9.8 mg/m³
LOEL: 10.2 mg/m³
Method: Other: International Research and Development Corporation Protocol, 1979
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.2%
Method: Four groups of young adult male and female rats (5/sex/dose) were exposed to zero, low, medium, and high concentrations of the test article as a fine dust. Observations were made with respect to the incidence of mortality, reactions displayed and body weight effects. Hematologic and clinical chemistry studies and urinalyses were conducted on all test and control animals on the day following final exposure. All animals were subjected to a gross necropsy. A complete set of organs and tissues were removed from each animal and preserved. Histopathologic studies were conducted on selected tissues and organs from the control and high-concentration groups. Weights of selected organs were recorded and subjected to statistical analyses. Body weights, hematological, biochemical, and urinalysis parameters and absolute and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-Test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge the significance of differences.

Results: All animals survived until terminal sacrifice. Exposure to the test article caused slight irritation in exposed animals. Slight body weight reductions and reductions in lung weights were observed in male rats in the high-concentration group. Histopathologic examination revealed no alterations in the tissues of the high-dose males or females as compared to controls. Slight depressions in blood glucose and elevations in SGOT values were noted in rats at all exposure levels, but these findings were not associated with the presence of any tissue lesions.

Reference: (31) Monsanto IR-78-96, International Research & Development Corp., 1979

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

Species/strain: Rat, Charles River CD
Sex: Male/Female
Route of Administration: Dietary
Exposure period: 2 years
Frequency of treatment: Daily
Post-exposure observation: None
Doses: 0, 5, 50, or 400 mg/kg/day (50/sex/dose)
Control group: Yes, concurrent no treatment
Method: Other – Hazleton Laboratories Europe Protocol, 1979
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity 96%
Method: The test substance was evaluated for chronic toxicity in a two-year chronic feeding study in male and female rats. The animals were exposed to concentrations of the test material in the diet equivalent to 0, 5, 50, or 400 mg/kg/day. The test compound was fed at the above doses to groups of 200 male and 200 female rats (50/sex/dose) over a two-year period, beginning when the males were 28 days old and the females 29 days old. The diet samples were analyzed via HPLC to determine actual dose levels and stability of the test material on the rat feed. Animals were observed twice daily during the first week of the study for signs of toxicity, and then at daily intervals thereafter. All animals were subjected to a necropsy. Major organs and tissues were examined for gross lesions. Organ weights were recorded for adrenals, brain, heart, liver, spleen, kidneys, gonads, lungs, and thyroids. Body weight, food consumption, behavior, hematology, blood chemistry and urinalysis results were recorded throughout the study. Complete gross necropsies were conducted on all animals found dead, on all animals sacrificed *in extremis*, and on all remaining animals at 24 months.

Results: There were no deaths that were attributed to exposure to the test material during the course of this study. Both males and females in the 50 and 400 mg dose groups exhibited statistically significant reductions in both body weight gain and food consumption as compared to control animals. Dose-related increases in kidney and liver weights were also observed in these two dose levels. Histopathologic examination of the tissues and organs taken from animals of the control group and the high- and mid-dose groups at sacrifice revealed no treatment-related lesions. Under the conditions of this test, no evidence of chronic toxicity was found.

Reference: (33) Monsanto HL-79-39, Hazelton Laboratories, 1979
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

***5.5 GENETIC TOXICITY IN VITRO**

A. BACTERIAL TEST

Type: Bacterial Reverse Gene Mutation

System of testing: *Salmonella typhimurium* TA-1535, TA-1537, TA-1538, TA-98, TA-100 and *Escherichia coli* WP₂ uvrA⁻

Concentration: 0.5 µg to 5,000 µg/plate (duplicate)

Metabolic activation: With and without

Results:

 Cytotoxicity conc: 1,000 µg/plate and above

 Genotoxic effects:

 With metabolic activation: Negative

 Without metabolic activation: Negative

Method: OECD Guideline 471, Genetic Toxicology: *Salmonella typhimurium* Reverse Mutation Assay, 1979

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 90-95%

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The methods are described in Hinderer et al., 1983 as follows:

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 were obtained from Dr. Bruce Ames. All indicator strains were kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine (Ames, 1980). In addition, the plates with the plasmid-carrying *Salmonella* strains (TA-98 and TA-100) were supplemented with 26µg/ml of ampicillin to ensure stable maintenance of the plasmid pKM101.

The bacterial strains were cultured at 37°C in Oxid Media #2 (nutrient broth), and Vogel Bonner Medium E with 2% glucose was used as the selective medium (Vogel and Bonner, 1956). The overlay agar was prepared according to the method of Ames et al (1975). S-9 liver homogenates, which were prepared from Aroclor 1254-induced and noninduced adult Sprague-Dawley male rats as described by Ames et al (1975, were prepared from Bionetics Laboratory Products, Litton Bionetics, Inc. An S-9 mix was prepared by adding the following ingredients per milliliter of mix: 4 µmoles NADP (sodium salt), 5 µmoles D-glucose-6-phosphate, 8 µmoles MgCl₂, 33 µmoles KCL, 100 µmoles sodium phosphate buffer (pH 7.4), and 100 µl of rat liver S-9 fraction.

All tests were based on the methods of Ames et al (1975). Test compounds were dissolved in dimethylsulfoxide (DMSO). Solvent and positive controls were as follows: Positive controls for the non-activation assays were 1 ug/plate sodium azide for TA-1535 and TA-100, 50 ug/plate 9-aminoacridine for TA-1537, 10 µg 2-nitrofluorene for TA-1538 and TA-98. The positive control used for the activation assays was 2.5 ug/plate 2-anthramine.) “The highest dose was established as one which produced some toxicity.

Criteria which were used to determine whether a chemical was mutagenic were: 1) an increase in revertants in strains TA-1535, TA-1537, TA-1538

of three times the solvent control; 2) an increase in revertants in strains TA-98 and TA-100 of twice the solvent control; 3) reproducibility; and 4) a dose-related response, and a consistent pattern of response between strains derived from the same parental strain.

The *E. coli* strain WP₂ uvrA⁻ was obtained from Dr. M.H.L. Green, MRC Cell Mutation Unit, University of Sussex, England. The indicator strain was kept at 4°C on standard methods agar plates or minimal medium plates supplemented with an excess tryptophan. Laboratory cultures were grown at 37°C in Oxoid #2 (nutrient broth). Vogel Bonner medium E (Vogel and Bonner, 1956) with 2% glucose was used as the selective medium. The overlay agar was prepared according to the method of Green and Murie (1976). The S-9 activation system was prepared as described for the *Salmonella* plate assay. The procedures were based on a modification of the methods described by Ames et al (1975).

DMSO was also used as the solvent to dissolve the test material. For the non-activation test 10 µg of methylnitrosoguanidine was the positive control. For the activation test 2.5 µg of 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide was the positive control.

A chemical was considered mutagenic if there was a dose-related response over a minimum of three test concentrations.

Note: A GLP- signed quality assurance statement was provided.

Result: The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: (33) Hinderer et al., Environmental Mutagenesis, 1983

Reliability: (1) Valid without restrictions. Meets generally accepted scientific method and is described in sufficient detail

Flag: Critical study for SIDS endpoint

Type: DNA Repair Test

System of testing: *Escherichia coli* W3110 (*pol A*⁺) and W3078 (*pol A*⁻)

Concentration: 0.5 – 2500 ug/plate (triplicate)

Metabolic activation: With and Without

Results:

 Cytotoxicity conc: With metabolic activation: Not determined (>2500 ug/plate)
 Without metabolic activation: Not determined (>2500 ug/plate)

 Precipitation conc: No data

 Genotoxic effects: With metabolic activation: Negative
 Without metabolic activation: Negative

Method: Rosenkranz and Leifer, Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient *E. coli*, 1980

Ames Mutagenicity Plate Test (Overlay Method) 1975

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
Other: Recrystallized 4x, purity 99+%

Remarks: The *E. coli* strains used were obtained from Dr. H. Rosenkranz, Columbia University, NY. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. Positive control chemicals were Methylmethane sulfonate (10ul/plate, nonactivation) and Dimethylnitrosamine (100 ul/plate, activation). The solvent used, DMSO, was also the negative control. The survival index was obtained by dividing the percent survival of the *pol A-* culture by the % survival of the *pol A+* culture. A survival index below 0.85 indicated a DNA-modifying effect. The positive controls exhibited the expected reductions of the survival index. The test material exhibited some slight reductions in the survival index in this test, but this was not considered significant due to the lack of dose response phenomena.

Result: Overall, the treatment did not induce preferential killing of the DNA repair deficient *pol A-* strain, indicating that the test compound was not genotoxic under test conditions.

Reference: (34) Litton Bionetics for CMA final report, 1981

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Type: Ames Bacterial Reverse Gene Mutation

System of testing: *Salmonella typhimurium* TA-1535, TA-1537, TA-1538, TA-98, TA-100

Concentration: 0.1, 1.0, 10, 100 or 500 ug/plate

Metabolic activation: With and Without

Results:

 Cytotoxicity conc: With metabolic activation: 500 ug/plate
 Without metabolic activation: 500 ug/plate

 Genotoxic effects:

 With metabolic activation: Negative
 Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Test (Overlay Method) 1975

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: 97 %

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were methylnitrosoguanidine (MNNG), 2-nitrofluorene (NF) and quinacrine mustard (QM). Positive control chemicals used for the

activation assays were 2-anthramine (ANTH), 2-acetylaminofluorine (AAF) and 8-aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: (35) Monsanto BIO-76-180, Litton Bionetics, November 1976
Reliability: (1) Valid without restriction

Type: Ames Bacterial Reverse Gene Mutation
System of testing: *Salmonella typhimurium* TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration: 1, 10, 100, 500, 1000, 2500 ug/plate (duplicate)
Metabolic activation: With and Without

Results:
Cytotoxicity conc: With metabolic activation: 1000 ug/plate
Without metabolic activation: 500 ug/plate

Precipitation conc: No data

Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Test (Overlay Method) 1975

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
Other: Recrystallized 4x, purity 99+%

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 1 ug/plate sodium azide, 10 ug/plate 2-nitrofluorene (NF) or 50 ug/plate 9-aminoacridine (9AA). Positive control used for the activation assays was 2.5 ug/plate 2-anthramine (ANTH). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: (34) Litton Bionetics for CMA final report, 1981

Reliability: (1) Valid without restriction

Type: Ames Bacterial Reverse Gene Mutation
System of testing: *Salmonella typhimurium* TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration: 0.1 to 1000 ug/plate (duplicate)
Metabolic activation: With and without
Results:

Cytotoxicity conc: ≥ 100 ug/plate
 Genotoxic effects:
 With metabolic activation: Negative
 Without metabolic activation: Negative
 Method: OECD Guideline 471, Genetic Toxicology: *Salmonella typhimurium*
 Reverse Mutation Assay, 1979
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: $> 99\%$
 Remarks: The standard Ames protocol was followed, with one exception. Instead of duplicate plates for the positive controls in trial #1, one of the plates contained twice the standard dose.
 Result: The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
 Reference: (36) Kurtz and Riddle, 1981 Unpublished
 Reliability: (1) Valid without restrictions. Meets generally accepted scientific method and is described in sufficient detail

B. NON-BACTERIAL IN VITRO TEST

Type: Mammalian Cell Forward Gene Mutation Assay
 System of testing: Mouse lymphoma cells L5178Y TK+/-
 Concentration: 0.156, 0.625, 2.5, 7.5, 15.0 $\mu\text{g/ml}$ without activation (triplicate)
 2.5, 10, 20, 40, 50 $\mu\text{g/ml}$ with activation (triplicate)
 Metabolic activation: With and without
 Results:
 Cytotoxicity conc: With metabolic activation: 40 $\mu\text{g/ml}$
 Without metabolic activation: 15 $\mu\text{g/ml}$
 Precipitation conc: None – soluble at all concentrations in acetone
 Genotoxic effects:
 With metabolic activation: Positive
 Without metabolic activation: Negative
 Method: Other: Clive, D. and Spector, J.F.S. (1975)
 Mutation Res. 31, 17-29
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
 Other: Recrystallized 4x, purity 99+%
 Results: The test substance was dissolved in acetone at 5 g/l. Stocks were prepared and serially diluted in acetone. The stocks were then diluted 1:100 into tubes of culture media containing the cells to initiate the treatments. The test compound appeared to be soluble in acetone over the entire range of concentrations selected. Concentrations greater than 40 $\mu\text{g/ml}$ proved to be highly cytotoxic in the presence of a rat liver S-9 preparation activation system, and cytotoxic at 15 $\mu\text{g/ml}$ without activation. Acetone (1%) was used as the solvent control substance. Growth medium without the addition of solvent was used as a negative control. No genetic effects were attributed to the presence of the solvent. EMS (0.5 $\mu\text{l/ml}$) and DMN (0.3 $\mu\text{g/ml}$) were used as reference mutagens

and induced mutation frequencies within the expected range. The test material induced significant increases in the TK locus in L5178Y mouse lymphoma cells only in the presence of S9 activation. The test material induced positive responses at two highly toxic treatments (40 and 50 ug/ml, percent relative growths 17% and 10.9%, respectively). In the absence of microsomal activation, moderately toxic concentrations up to 7.5 ug/ml were not mutagenic. Therefore, the test compound was considered to be active only in the presence of activation in this assay.

Non-Activation Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	---	44.0	252.0	17.5
Negative Control	---	49.0	289.0	17.0
EMS	0.5 µl/ml	321.0	126.0	254.8
Test Cpd.	0.156	47.0	307.0	15.3
	0.625	36.0	220.0	16.4
	2.5	24.0	189.0	12.7
	7.5	24.0	194.0	12.4
	15.0	7.7	112.0	39.3

Activation with S-9 Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	---	32.0	240.0	13.3
Negative Control	---	48.0	223.0	21.5
DMN	0.3 µl/ml	39.0	15.0	260.0
Test Cpd.	2.5	38.0	154.0	24.7
	10.0	52.0	139.0	37.4
	20.0	57.0	210.0	27.1
	40.0	71.0	110.0	64.5
	50.0	65.0	122.0	53.3

Reference: (34) Litton Bionetics for CMA final report, 1981

Reliability: (1) Valid without restriction

Type: Mammalian Cell Forward Gene Mutation Assay

System of testing: Mouse lymphoma cells L5178Y TK+/-

Concentration: 0.78, 1.56, 3.13, 6.25, 12.5 µg/ml without activation (duplicate)

1.56, 3.13, 6.25, 12.5, 25.0 µg/ml with activation (duplicate)

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 50 µg/ml

Without metabolic activation: >12.5 µg/ml

Precipitation conc: 300 ug/ml (in DMSO)

Genotoxic effects:

With metabolic activation: Weak Positive

Without metabolic activation: Negative

Method: Other: Clive, D. and Spector, J.F.S. (1975)

Mutation Res. 31, 17-29

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 96%

Results:

The test substance was dissolved in DMSO at 25 mg/ml. Stocks were prepared and serially diluted in DMSO. The stocks were then diluted 1:100 into tubes of culture media containing the cells to initiate the treatments. A preliminary cytotoxicity experiment preceded the mouse lymphoma assay. Concentrations greater than 50 µg/ml proved to be highly cytotoxic in the presence of a rat liver S-9 preparation activation system, and cytotoxic above 12.5 µg/ml without activation. DMSO (1%) was used as the solvent control substance. Growth medium without the addition of solvent was used as a negative control. No genetic effects were attributed to the presence of the solvent. EMS (0.5 µl/ml) and DMN (0.3 µg/ml) were used as reference mutagens and induced mutation frequencies within the expected range. The test material induced a slight, but significant, increase in the TK locus in L5178Y mouse lymphoma cells only in the presence of S9 activation, and only at the highest dose tested. In the absence of microsomal activation, moderately toxic concentrations up to 12.5 µg/ml were not mutagenic. Therefore, the test compound was considered to be weakly active only in the presence of activation in this assay.

Non-Activation Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	---	73	238	30.7
Negative Control	--	69	254	27.2
EMS	0.5 µl/ml	549	111	494.6
Test Cpd.	0.78	90	202	44.6
	1.56	89	232	38.4
	3.13	127	225	56.4
	6.25	78	196	39.8
	12.5	69	167	41.3

Activation with S-9 Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	---	143	181	79.0
Negative Control	---	155	274	56.6
DMN	0.3 µl/ml	49	9	544.4
Test Cpd.	1.56	146	289	50.5
	3.13	190	252	75.4
	6.25	172	256	67.2
	12.50	152	317	47.9
	25.00	242	130	186.2

Reference: (37) Monsanto BO-78-223, Litton Bionetics, 1979

Reliability: (1) Valid without restriction

Type: Mammalian Cell Transformation Assay

System of testing: BALB/3T3 cells

Concentration: 30.0 µg/ml – 0.75 µg/ml

Metabolic activation: Without

Results:

Cytotoxicity conc: 31.3 µg/ml

Precipitation conc: >0.25 mg/ml

Genotoxic effects: Negative

Method: Kakunaga , T., A Quantitative System for Assay of Malignant Transformation by Chemical Carcinogens using a Clone Derived from BALB/3T3, 1973

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
Other: Recrystallized 4x, purity 99+%

Remarks: The I(13) BALB/3T3 mouse cell clones for this study were obtained from Dr. Kakunaga of the National Cancer Institute. Further subclones, selected for low spontaneous frequencies of foci formation, were used for the assay. DMSO was used for the solvent and the solvent (negative) control. A known carcinogen, 3-methylcholanthrene (MCA, 5 ug/ml) was used as the positive control. A preliminary cytotoxicity test determined the concentrations selected for the transformation assay (30 ug/ml – 0.75 ug/ml, corresponding to a survival range of 20-90%). Under the conditions of this assay, the test substance did not induce a significant increase in transformed foci over the applied concentration range.

Reference: (34) Litton Bionetics for CMA final report, 1981

Reliability: (1) Valid without restriction

Type: Mammalian Cell Cytogenetics Assay – Sister Chromatid Exchange

System of testing: CHO Cells, clone K1-BH4

Concentration: 1, 5, 10, 20, or 40 ug/ml without metabolic activation (duplicate)
5, 10, 15, 30 or 60 ug/ml with metabolic activation (duplicate)

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 100 ug/ml
Without metabolic activation: 50 ug/ml

Precipitation conc: None

Genotoxic effects:

With metabolic activation: Negative

Without metabolic activation: Negative

Method: A quantitative assay of mutation induction at the HG/PRT locus in Chinese hamster ovary cells, Mutation Research 45: 91-101
O'Neil et al., 1977; OECD 479

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
Other: Recrystallized 4x, purity 99+%

Remarks: The Chinese hamster ovary cells used for this cytogenetics assay were acquired from the Oak Ridge National Laboratories, TN. An Aroclor 1254-induced rat liver homogenate preparation (S-9) was the activation system. The solvent used for concentration solutions and for the solvent control was DMSO. The positive control for the assay with S9 was Dimethylnitrosamine (DMN) at 100 mg/ml of medium. The positive control for the assay without S9 was Ethylmethanesulfonate (EMS) at 200 ug/ml of medium. In a preliminary cytotoxicity assay, the relative cell survival decreased from 74% at 30 ug/ml to 43% at 50 ug/ml in non-activated cultures, and from 80% at 10 ug/ml to 23% at 100 ug/ml in activated cultures. Test cells were treated for 5 hours, washed 3 times, and then incubated for 19 hours. The

mutant frequency was calculated by correcting the total number of mutant clones by the cloning efficiency of the cells at the time of mutant selection. Dose-response analyses were performed on the transformed mutant frequency data by the one-way analysis of variance method (Snee and Irr, 1981). A statistically significant (t-test, $p \leq 0.05$) but less than 2-fold increase in SCE frequency was observed at 20 and 40 ug/ml in the non-activated tests, and at 30 and 60 ug/ml in the activated tests.

Results: Dose response was not observed in any experiment. Therefore, the results were interpreted as non-mutagenic under the conditions of this assay.

Reference: (38) Pharmakon Research International for CMA, 1984

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Type: Mammalian Cell Cytogenetics Assay – Sister Chromatid Exchange

System of testing: CHO Cells, clone K1-BH4

Concentration: 5 to 75 ug/ml without metabolic activation (duplicate)
5 to 100 ug/ml with metabolic activation (duplicate)

Metabolic activation: With and without

Results:

 Cytotoxicity conc: With metabolic activation: No data
 Without metabolic activation: No data

 Precipitation conc: None

 Genotoxic effects: With metabolic activation: Negative
 Without metabolic activation: Negative

Method: A quantitative assay of mutation induction at the HG/PRT locus in Chinese hamster ovary cells, Mutation Research 45: 91-101
O'Neil et al., 1977; OECD 479

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 95-97% (2 commercial grades)
Other: Recrystallized 4x, purity 99+%

Remarks: The Chinese hamster ovary cells used for this cytogenetic assay were acquired from the Oak Ridge National Laboratories, TN. An Aroclor 1254-induced rat liver homogenate preparation (S-9) was the activation system. The solvent used for concentration solutions and for the solvent control was Acetone. The positive control for the assay with S9 was Dimethylnitrosamine (DMN) at 100 mg/ml of medium. The positive control for the assay without S9 was Ethylmethanesulfonate (EMS) at 200 ug/ml of medium. Test cells were treated for 5 hours, washed 3 times, and then incubated for 19 hours. The mutant frequency was calculated by correcting the total number of mutant clones by the cloning efficiency of the cells at the time of mutant selection. Dose-response analyses were performed on the transformed mutant frequency data by the one-way analysis of variance method (Snee and Irr, 1981). A statistically significant (t-test, $p \leq 0.05$) but less than 2-fold increase in SCE frequency was observed at 50 and 75 ug/ml in the non-activated tests, and at all doses except 5 ug/ml in the activated tests. The effect was dose-related in the presence of activation. The

relative cell survival was observed to range from 65-82% in non-activated cultures and from 66-82% in activated cultures during the experiment.

Result: Due to the lack of two-fold doubling, the results were interpreted as non-mutagenic under the conditions of this assay.
Reference: (38) Pharmakon Research International for CMA, 1984
Reliability: (1) Valid without restriction

Type: Mammalian Cell Cytogenetics Assay – Sister Chromatid Exchange
System of testing: CHO Cells, clone K1-BH4
Concentration: 5, 20, 40, 60, 80 ug/ml without metabolic activation (duplicate)
10, 40, 80, 120, 240 ug/ml with metabolic activation (duplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 150 ug/ml
Without metabolic activation: 50 ug/ml
Precipitation conc: None
Genotoxic effects: With metabolic activation: Negative
Without metabolic activation: Negative
Method: A quantitative assay of mutation induction at the HG/PRT locus in Chinese hamster ovary cells, Mutation Research 45: 91-101
O'Neil et al., 1977; OECD 479
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 95-97%
Remarks: The Chinese hamster ovary cells used for this cytogenetic assay were acquired from the Oak Ridge National Laboratories, TN. An Aroclor 1254-induced rat liver homogenate preparation (S-9) was the activation system. The solvent used for concentration solutions and for the solvent control was acetone. The positive control for the assay with S9 was Dimethylnitrosamine (DMN) at 100 mg/ml of medium. The positive control for the assay without S9 was Ethylmethanesulfonate (EMS) at 200 ug/ml of medium. Test cells were treated for 5 hours, washed 3 times, and then incubated for 19 hours. The mutant frequency was calculated by correcting the total number of mutant clones by the cloning efficiency of the cells at the time of mutant selection. Dose-response analyses were performed on the transformed mutant frequency data by the one-way analysis of variance method (Snee and Irr, 1981).
Result: A statistically significant (t-test, $p < 0.05$) increase in mutant frequency was not observed at any dose level in the presence or absence of activation.
Reference: (38) Pharmakon Research International for CMA, 1984
Reliability: (1) Valid without restriction

Type: Mammalian Cell Cytogenetics Assay – Sister Chromatid Exchange
System of testing: CHO cells
Concentration: 0.625, 1.25, 2.5, 5, 7.5 and 10 ug/ml (duplicate)
Metabolic activation: With and without
Results:

Cytotoxicity conc: With metabolic activation: None
 Without metabolic activation: None
 Precipitation conc: None
 Genotoxic effects: With metabolic activation: Negative
 Without metabolic activation: Negative
 Method: A quantitative assay of mutation induction at the HG/PRT locus in Chinese hamster ovary cells, Mutation Research 45: 91-101
 O'Neil et al., 1977
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: 95-97%
 Remarks: Based on a preliminary toxicity screen, both activated and non-activated cultures were treated with doses of the test article ranging from 0.625–10 ug/ml. An Aroclor 1254-induced rat liver homogenate preparation (S-9) was the activation system. The solvent used for concentration dilutions and for the solvent control was acetone. The positive control for the assay with S9 was 0.00025 M Cyclophosphamide. The positive control for the assay without S9 was Triethylenemelamine at 0.75 ug/ml of medium. Test cells were treated for 2 hours, washed 3 times, and then incubated for 17 hours. Colcemid was added for the last three hours of incubation. Fifty cells per treatment were analyzed for chromosomal aberrations. The mutant frequency was calculated by correcting the total number of mutant clones by the cloning efficiency of the cells at the time of mutant selection. Dose-response analyses were performed on the transformed mutant frequency data by the one-way analysis of variance method (Snee and Irr, 1981). The positive controls produced the expected large increases in the frequency of chromosomal aberrations. A statistically significant (t-test, p<0.05) increase in mutant frequency was not observed at any dose level of the test substance in the presence or absence of activation.
 Reference: (39) Litton Bionetics/BF Goodrich, 1979
 Reliability: (1) Valid without restriction

* 5.6 GENETIC TOXICITY IN VIVO

Type: Dominant Lethal Assay
 Species/strain: Rat, Sprague-Dawley
 Sex: Male
 Route of Administration: Gavage
 Exposure period: 56 days
 Doses: 0, 125, 250 or 500 mg/kg bw/day
 Results:
 Effect on mitotic index or P/N ratio: Negative
 Genotoxic effects: Negative
 Method: Other: Food and Drug Research Labs Protocol, 1980
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: 90-95%

Remarks: The test substance was evaluated for genotoxicity in the dominant lethal test in groups of 10 male rats treated by gavage at the above dose levels. Following treatment, each male rat was housed with two virgin female rats/week for two weeks. The females were sacrificed thirteen days after mating for determination of dominant lethal effects. The treatment had no adverse effects with respect to clinical signs, mortality rate, body weight gain, or organ weights of adult male rats. The treatment also had no effect on pregnancy, early fetal death, implantation, or preimplantation losses in the females. Positive control treatment with a single intraperitoneal injection of 0.25 mg/kg bw triethylenemelamine produced the expected dominant lethal effects. Under the conditions of this assay, the test compound results were negative, indicating that the compound was not mutagenic to the germ cells of male rats.

Reference: (40) BF Goodrich/Food and Drug Research Laboratories, 1980
Reliability: (1) Valid without restriction
Flag: Critical study for SIDS endpoint

Type: Modified Dominant Lethal Assay
Species/strain: Rat
Sex: Male and female
Route of Administration: Gavage
Exposure period: 2 days prior to mating for both males and females
Doses: 200 mg/kg bw/day
Results:
Effect on mitotic index or P/N ratio: No data
Genotoxic effects:
Method: Other, 1980
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: The test substance was evaluated in a modified dominant lethal assay in which 200 mg/kg was administered to both male and female rats two days prior to mating. An increase in total embryonic mortality, reduced fetal body weights, and a delay in the onset of conception were reported.

Reference: (41) Aleksandrov, S.E. Byull. Eksp. Biol. Med., 1982
Reliability: (4) Not assignable - data from a secondary literature source

5.7 CARCINOGENICITY

Species/strain: Rat, Charles River CD
Sex: Male/Female
Route of Administration: Dietary
Exposure period: 2 years
Frequency of treatment: Daily for
Post-exposure observation: None
Doses: 0, 5, 50 or 400 mg/kg/day (50/sex/dose)
Control group: Yes, concurrent no treatment

Results: Negative
 Method: Other – Hazleton Laboratories Europe Protocol, 1979
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity 96%
 Method: The test compound was fed at the above doses to groups of 200 male and 200 female rats over a two-year period, beginning when the males were 28 days old and the females 29 days old. The diet samples were analyzed via HPLC to determine actual dose levels and stability of the test material on the rat feed. Animals were observed twice daily during the first week of the study for signs of toxicity, and then at daily intervals thereafter. Body weight, food and water consumption were recorded weekly. All animals were subjected to a necropsy. Major organs and tissues were examined for gross lesions. Organ weights were recorded for adrenals, brain, heart, liver, spleen, kidneys, gonads, lungs and thyroids. Body weight, food consumption, behavior, hematology, blood chemistry and urinalysis results were recorded throughout the study. Complete gross necropsies were conducted on all animals found dead, on all animals sacrificed *in extremis*, and on all remaining animals at 24 months.

Results: The test substance was evaluated for oncogenicity in a two-year chronic feeding study in male and female rats. 200 animals (50/sex/dose) were exposed to concentrations of the test material in the diet equivalent to 0, 5, 50 or 400 mg/kg/day. There were no deaths that were attributed to exposure to the test material during the course of this study. Both males and females in the 50 and 400 mg/kg dose groups exhibited statistically significant reductions in both body weight gain and food consumption as compared to control animals. Dose-related increases in kidney and liver weights were also observed in these to dose levels. Histopathologic examination of the tissues and organs taken from animals of the control group and the high- and mid-dose groups at sacrifice revealed no treatment-related lesions. Microscopic examinations of any suspect neoplasms were conducted on all sacrificed animals and any animal that died during the study. No differences were noted between test and control rats as to the organ system involved, type or classification of neoplasms. The spectrum of neoplasms observed compared favorably to the historical data at this laboratory for rats of this age and strain.

Conclusion: Under the conditions of this test, no evidence of oncogenicity was found.
 Reference: (32) Monsanto HL-79-39, Hazelton Laboratories, 1979
 Reliability: (1) Valid without restriction

Species/strain: Mice, B6C3F1 and B6AKF1
 Sex: Male/Female
 Route of Administration: Oral gavage on days 7-28, oral feed for remainder or single subcutaneous injection on Day 28
 Exposure period: 79 weeks
 Frequency of treatment: Daily for feed study, once for injection study
 Post-exposure observation: 78 weeks for injection, none for intubation/feed
 Doses: Gavage = 215 mg/kg bw
 Feed = 90 mg/kg/day
 Injection = 1000 mg/kg

Control group: Yes
 Other: Positive Control

Result: Negative

Method: Litton Bionetics Research Labs Protocol, 1968

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity 96%

Remarks: Study was undertaken to determine the carcinogenic potential of 130 chemicals that had been used in the formulations of insecticides, herbicides and fungicides.

Method: In a National Cancer Institute study, 18 virgin male and 18 virgin female mice from two hybrid strains were dosed with the test substance. Two types of studies were run simultaneously. One group of 36 mice received a single subcutaneous injection administered in the nape of the neck at the 28th day of age, with no exposure to the test substance thereafter. The second group of 26 mice received a daily oral gavage dose of the test article administered from the 7th to 28th days of age, and then daily in their feed mix thereafter.

Results: All compounds administered orally as positive controls were carcinogenic, while only two of the positive controls (urethane, ethyleneimine) administered subcutaneously had carcinogenic activity. There were no findings of carcinogenic effects attributed to the test substance in either 79-week study.

Reference: (42) Litton Bionetics/NCI Report # PB223-159 (1968)

Reliability: (2) Valid with restrictions - Intubation/feed part of this study followed generally accepted parameters for a 1968 carcinogenicity assessment, but not all test parameters comply with current guidelines. No GLP data. The reliance on a single subcutaneous injection as adequate for the other portion of this study is questionable.

*5.8 TOXICITY TO REPRODUCTION

Type: Fertility

Species/strain: Rats

Sex: Male

Route of Administration: Gavage

Exposure period: 56 days

Frequency of treatment: Daily, 7 days/week

Post exposure observation period: 2 weeks

Doses: 0, 125, 250 or 500 mg/kg/day

Control group: Yes, Concurrent vehicle

Method: Green et al. (1974) Toxicol. Appl. Pharmacol 39:549-552; Russel and Matter (1980) Mutat. Res. 75:279-302.

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >95%

Method: The test substance was evaluated for genotoxicity in the dominant lethal test in groups of 10 male rats treated by gavage at the above dose levels. Following treatment, each male rat was housed with two

virgin female rats/week for two weeks. The females were sacrificed thirteen days after mating for determination of dominant lethal effects.

Results: The treatment had no adverse effects with respect to clinical signs, mortality rate, body weight gain, or organ weights of adult male rats. The treatment also had no effect on pregnancy, early fetal death, implantation, or preimplantation losses in the females. Positive control treatment with a single intraperitoneal injection of 0.25 mg/kg bw triethylenemelamine produced the expected dominant lethal effects. No evidence of reduced fertility was observed. Treated animals had similar conception rates to those of controls. Mean body weight and mean body weight gain did not reveal any statistically significant effect in any of the dose groups. Tissue-to-body weight ratios were normal, with the exception of an increase in the stomach/body weight ratio in the 125 and 250 mg/kg dose groups. No abnormalities were noted in selected animals during gross pathological examination.

Conclusion: Under the conditions of this assay, the test compound results were negative, indicating that the compound was not mutagenic to the germ cells of male rats.

Reference: (43) Hinderer, et al., Tox. Appl. Pharm., 1982

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Species/strain: Rat, Charles River CD

Sex: Male/Female

Route of Administration: Dietary

Exposure period: 2 years

Frequency of treatment: Daily for

Post-exposure observation: None

Doses: 0, 5, 50 or 400 mg/kg/day (50/sex/dose)

Control group: Yes, concurrent no treatment

Results: Negative

Method: Other – Hazleton Laboratories Europe Protocol, 1979

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity 96%

Method: The test compound was fed at the above doses to groups of 200 male and 200 female rats over a two-year period, beginning when the males were 28 days old and the females 29 days old. The diet samples were analyzed via HPLC to determine actual dose levels and stability of the test material on the rat feed. Animals were observed twice daily during the first week of the study for signs of toxicity, and then at daily intervals thereafter. Body weight, and food and water consumption were recorded weekly. All animals were subjected to a necropsy. Major organs and tissues were examined for gross lesions. Organ weights were recorded for gonads, and other organs. Behavior, hematology, blood chemistry and urinalysis results were recorded throughout the study. Complete gross necropsies were conducted on all animals found dead, on all animals sacrificed *in extremis*, and on all remaining animals at 24 months.

Results: Histopathologic examination of the tissues and organs taken from animals of the control group and the high- and mid-dose groups at sacrifice revealed no treatment-related lesions.

Conclusion: Under the conditions of this test, no effects on reproductive organs were found.

Reference: (32) Monsanto HL-79-39, Hazelton Laboratories, 1979

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Type: Fertility

Species/strain: Rats

Sex: Male and female

Route of Administration: Gavage

Exposure period: 3 days

Frequency of treatment: 1st and 3rd days of estrus (female); twice at intervals of 3 days

Post exposure observation period: Until 19th day of pregnancy

Premating exposure period: No data

Duration of the test: No data

Doses: 0 or 200 mg/kg bw

Control group: Yes, Concurrent vehicle

Results: There were no visible signs of poisoning in the parent animals. Changes in the estrous cycle were noted, as well as delays in conception in treated females. Decreased fetal weights were observed. There was an increase in total embryonic mortality, but postimplantation embryonic mortality was within the normal range.

Method: No data

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: >95%

Remarks: Embryonic mortality study

Reference: (44) Aleksandrov, S.E., Byull. Eksp. Biol. Med., 1982

Reliability: (4) Not assignable - data from a secondary literature source

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain: Rat, Charles River COBS CD

Sex: Female

Route of Administration: Oral gavage

Duration of the test: Day 6-15 of gestation

Exposure period: 10 days

Frequency of treatment: Daily

Doses: 0, 100, 300 or 1000 mg/kg/day

Control group: Yes
Concurrent vehicle

NOEL Maternal Toxicity: 100 mg/kg/day

NOEL Teratogenicity : 1000 mg/kg/day

Method: Other: Wilson, J.G., Embryological Considerations in Teratology, Teratology – Principles and Techniques, 1965

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >96%

Method: One hundred female rats, age 80-120 days old, were mated for this study. Pregnant rats were used to determine the teratogenic potential of the test substance. The above doses were administered orally by gavage as a single daily dose on days 6-15 of gestation at a constant volume of 10 ml/kg. The control group received the vehicle only (Mazola corn oil) on a comparable regimen at a volume of 10 ml/kg. Dams were observed daily for mortality and overt changes in appearance and behavior. Caesarean sections were performed on all dams on day 20 of gestation. The uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and non-viable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed and individually numbered and tagged for identification. Half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor blade sectioning. The remaining fetuses were fixed in alcohol, macerated in potassium hydroxide, and dye-stained for subsequent skeletal analysis.

Results: Maternal general toxicity: One death occurred in the high-dosage group on gestation day 14, but this was attributed to a gavage error. All other animals survived until terminal sacrifice. A dose-related increase in matting and staining of the anogenital hair coat was noted in the 300 and 1000 mg/kg/day treatment groups, and by decreases in mean maternal body weight gain as well. There was also a slight increase in hair loss. Pregnancy/litter data: No biologically meaningful or statistically significant differences were observed in the mean numbers of *corporea lutea*, total implantations, viable fetuses, early or late resorptions, postimplantation loss, mean fetal body weights, or mean fetal sex distribution in the treated groups when compared to the control group. Fetal data: There were no biologically meaningful or statistically significant differences in the number of litters with malformations in the treated groups when compared to the control group. No biologically meaningful differences in the number of fetuses or litters with developmental or genetic variations were observed in the treated groups when compared to the control group.

Conclusion: The test article did not produce a teratogenic response when administered orally by gavage to pregnant rats at a dose level of 1000 mg/kg/day or less.

Reference: (45) Monsanto IR-78-102, International R & D Corp., 1981

Reliability: (1) Valid without restriction

Flag: Critical study for SIDS endpoint

Species/strain: Rat, Wistar

Sex: Female

Route of Administration: Oral feed

Duration of the test: 21 Days

Exposure period: Day 0 of gestation – Day 21 postparturition

Frequency of treatment: Daily

Doses: 0, 3.9, or 81 mg/kg/day

Control group: Yes, Concurrent no treatment

NOEL Maternal Toxicity: 81 mg/kg/day

NOEL Teratogenicity: 81 mg/kg/day

Results:

Maternal general toxicity: No toxic effects noted

Pregnancy/litter data: Slight increase in the stillborn litter number

Fetal data: No harmful effects on the fetuses with respect to external, skeletal or visceral anomalies. The postnatal development of the offspring was normal.

Method: No data

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity >95%

Remarks: None

Reference: (46) Morita, et al., 1981

Reliability: (4) Not assignable - data from a secondary literature source

Species/strain: Rat, Wistar

Sex: Female

Route of Administration: Oral feed

Duration of the test: 21 days

Exposure period: Day 0 – Day 21 of gestation

Frequency of treatment: Daily

Doses: 0, 13 or 270 mg/kg

Control group: Yes, Concurrent no treatment

NOEL Maternal Toxicity: 270 mg/kg

NOEL Teratogenicity: 270 mg/kg

Results:

Maternal general toxicity: No toxic effects noted

Pregnancy/litter data: No toxic effects noted

Fetal data: No harmful effects on the fetuses with respect to external, skeletal or visceral anomalies. The postnatal development of the offspring was normal.

Method: No data

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity >95%

Remarks: None

Reference: (46) Morita, et al., 1981

Reliability: (4) Not assignable - data from a secondary literature source

Species/strain: Rat

Sex: Female

Route of Administration: Gavage

Duration of the test: Until 11th day of pregnancy

Exposure period: 2 days

Frequency of treatment: 4th and 11th day of pregnancy

Doses: 200 mg/kg bw
 Control group: Yes
 NOEL Maternal Toxicity: Not determined
 NOEL Teratogenicity : Not determined
 Results: The object of this investigation was to study the level of embryonic mortality.
Maternal general toxicity: No visible signs of poisoning were noted.
Pregnancy/litter data: Increased incidence of total, preimplantation and postimplantation lethality
Fetal data: Slight decreased in fetal weights.
 Method: No data
 GLP: No data
 Test substance: As prescribed by 1.1-1.4, purity: >96%
 Remarks: None
 Reference: (44) Aleksandrov, S.E., Byull. Eksp. Biol. Med., 1982
 Reliability: (4) Not assignable - data from a secondary literature source

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Biochemical or cellular interactions
 Results: The reactions of representative amino acids lysine, cysteine and glycine with the test compound were examined. New compounds were confirmed between the test compound and all three amino acids. No reaction was observed with morpholine. The new compounds were examined spectroscopically. The conjugation between the test compound and cysteine was postulated as a reaction of the test compound's disulfide bond and the -SH and -NH₂ groups of the amino acid moieties.
 Reference: (47) Wang and Tabor, Contact Dermatitis, 1988
 Reliability: (4) Not assignable - data from a secondary literature source

B. Toxicodynamics, toxicokinetics

Type: Metabolism/Metabolites
 Results: The metabolic fate of the test article in rats was studied using tracer techniques. The compound, given orally to rats, was rapidly excreted in the urine and feces. Five urinary metabolites, (2-mercaptobenzothiazole, its three conjugates mercapturate, glucuronide and sulfate, and 2-mercaptobenzothiazole disulfide) were confirmed. 2-mercaptobenzothiazole disulfide was also found as a fecal metabolite. The test compound was partially transformed in the stomach to 2-mercaptobenzothiazole disulfide, which was predominately excreted into the feces. In the liver, the test compound was mainly transformed to 2-mercaptobenzothiazole and its conjugates. The S-glucuronide and S-sulfate conjugates were predominately excreted into the bile.
 Reference: (48) Fukuoka, et al., Archives of Toxicology, 1995
 Reliability: (4) Not assignable - data from a secondary literature source

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