

201-15978 B

# I U C L I D

## Data Set

05 JUL 29 AM 8:05

RECEIVED

**Existing Chemical** : ID: 19248-13-6  
**CAS No.** : 19248-13-6  
**Substance name** : N-ethyl-N-(3-methylphenyl)-1,2-ethanediamine  
**Molecular Formula** : C11H18N2

**Producer related part**  
**Company** : Eastman Chemical Company  
**Creation date** : 19.09.2003

**Substance related part**  
**Company** : Eastman Chemical Company  
**Creation date** : 19.09.2003

**Status** :  
**Memo** :

**Printing date** : 27.07.2003  
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**Chapter (profile)** : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10  
**Reliability (profile)** : Reliability: without reliability, 1, 2, 3, 4  
**Flags (profile)** : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE),  
Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

# 1. General Information

**Id** 19248-13-6  
**Date** 29.10.2003

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## 1.2 SYNONYMS AND TRADENAMES

**1,2-ethanediamine, N-ethyl-N-(3-methylphenyl)-**

23.10.2003

**N-(2-aminoethyl)-N-ethyl-m-toluidine**

23.10.2003

**Amine III**

23.10.2003

**N-ethyl-N-(3-methylphenyl)-1,2-ethanediamine**

23.10.2003

**N-ethyl-N-B-aminoethyl-m-toluidine**

## 1.3 IMPURITIES

**Purity** : typical for substance  
**CAS-No** : 91-67-8  
**EC-No** : 202-089-3  
**EINECS-Name** : N,N-diethyl-m-toluidine  
**Molecular formula** :  $C_{11}H_{17}N_2$   
**Value** : = 0 - 2 % w/w  
**Reliability** : (1) valid without restriction  
23.10.2003

(1)

## 1.4 ADDITIVES

## 1.5 TOTAL QUANTITY

### 1.6.1 LABELLING

### 1.6.2 CLASSIFICATION

### 1.6.3 PACKAGING

## 1.7 USE PATTERN

### 1.7.1 DETAILED USE PATTERN

## 1.7.2 METHODS OF MANUFACTURE

## 1.8 REGULATORY MEASURES

### 1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

### 1.8.2 ACCEPTABLE RESIDUES LEVELS

### 1.8.3 WATER POLLUTION

### 1.8.4 MAJOR ACCIDENT HAZARDS

### 1.8.5 AIR POLLUTION

### 1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

### 1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

### 1.9.2 COMPONENTS

### 1.10 SOURCE OF EXPOSURE

### 1.11 ADDITIONAL REMARKS

### 1.12 LAST LITERATURE SEARCH

### 1.13 REVIEWS

**2.1 MELTING POINT**

**Value** : < 0 °C  
**Decomposition** : no  
**Method** : other: measured  
**Year** : 2003  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : The material was placed in a -5 to -10 C freezer for one hour and did not freeze. The estimated melting point value using EPIWIN is 63.05 C.  
**Source** : Eastman Chemical Company, unpublished data  
**Reliability** : (2) valid with restrictions  
 Study was not performed according to GLP. Purity of the material was unknown but is typically >98%.  
**Flag** : Critical study for SIDS endpoint  
 27.07.2005

**2.2 BOILING POINT**

**Value** : > 250 °C at 1013 hPa  
**Decomposition** : yes  
**Method** : ASTM D1078  
**Year** : 2003  
**GLP** : No  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : The liquid did not boil at 250 degrees C, which was the highest temperature that could be recorded with the thermometer that was used. The material turned dark brown upon heating. The purity of the material was 98-100%.  
**Source** : Eastman Chemical Company, unpublished data  
**Reliability** : (2) valid with restrictions  
 Study was not performed according to GLP. Purity of the material was unknown but is typically >98%.  
**Flag** : Critical study for SIDS endpoint  
 23.10.2003

**2.3 DENSITY**

**Type** : relative density  
**Value** : = 0.982 g/cm<sup>3</sup> at 20 °C  
**Method** : other: measured  
**Year** :  
**GLP** : No  
**Test substance** : as prescribed by 1.1 - 1.4  
**Reliability** : (2) valid with restrictions  
 No study details given. Data provided by manufacturer's material safety data sheet.  
 23.10.2003 (1)

**2.3.1 GRANULOMETRY****2.4 VAPOUR PRESSURE**

**Value** : = 0.036 hPa at 25 °C

## 5. Toxicity

**Id** 19248-13-6  
**Date** 29.10.2003

**Method** : other (calculated) by EPIWIN MPBPwin  
**Year** : 2003  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : Inputs to the program were CAS No. 19248-13-6, a melting point of 0 and a boiling point of 250 degrees C.  
**Reliability** : (2) valid with restrictions  
Data were calculated using a model program.  
**Flag** : Critical study for SIDS endpoint  
23.10.2003 (6)

### 2.5 PARTITION COEFFICIENT

**Partition coefficient** : octanol-water  
**Log pow** : = 2.23 at 20 °C  
**pH value** : = 7  
**Method** : other (calculated)  
**Year** : 2003  
**GLP** : No  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : Inputs to the program were CAS No. 19248-13-6, a melting point of 0 and a boiling point of 250 degrees C.  
**Reliability** : (2) valid with restrictions  
Data were calculated using a model program.  
**Flag** : Critical study for SIDS endpoint  
23.10.2003 (5)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : water  
**Value** : = 12,090 mg/l at 20 °C  
**pH value** : = 7  
**concentration** : at °C  
**Temperature effects** :  
**Examine different pol.** :  
**pKa** : at 25 °C  
**Description** :  
**Stable** :  
**Remark** : Inputs to the program were CAS No. 19248-13-6, a melting point of 0 and a boiling point of 250 degrees C.  
**Reliability** : (2) valid with restrictions  
Data were calculated using a model program.  
**Flag** : Critical study for SIDS endpoint  
23.10.2003 (7)

### 2.6.2 SURFACE TENSION

### 2.7 FLASH POINT

### 2.8 AUTO FLAMMABILITY

**2.9 FLAMMABILITY****2.10 EXPLOSIVE PROPERTIES****2.11 OXIDIZING PROPERTIES****2.12 DISSOCIATION CONSTANT****2.13 VISCOSITY****2.14 ADDITIONAL REMARKS****3.1.1 PHOTODEGRADATION**

**Type** : air  
**Light source** : sun light  
**Light spectrum** : nm  
**Relative intensity** : based on intensity of sunlight  
**INDIRECT PHOTOLYSIS**  
**Sensitizer** : OH  
**Conc. of sensitizer** :  
**Rate constant** : = 0.0000000002513575 cm<sup>3</sup>/(molecule\*sec)  
**Degradation** : = 50 % after 0.5 hour(s)  
**Deg. product** :  
**Method** : other (calculated)  
**Year** : 2003  
**GLP** : No  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : Inputs to the program were CAS No. 19248-13-6, a melting point of 0 and a boiling point of 250 degrees C.  
**Reliability** : (2) valid with restrictions  
The data were obtained using a model estimation program.  
**Flag** : Critical study for SIDS endpoint  
23.10.2003 (4)

**3.1.2 STABILITY IN WATER**

**Test substance** : as prescribed by 1.1 - 1.4  
**Result** : EPIWIN HYDROWIN Program can estimate the hydrolysis rate constant for certain classes of organic compounds, but not amines.  
23.10.2003  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : The structural features of amine 3 for consideration of susceptibility to hydrolysis include an aromatic methyl group, an aromatic dialkyl-substituted tertiary amine, and a primary alkyl amine. Each of these three functionalities are well known to be stable to reaction with water under hydrolytic conditions. The requisite leaving groups are not sufficiently labile to be displaced by the nucleophilic attack of a water molecule, as is required in the mechanism of many hydrolysis reactions. CH<sub>3</sub>, NH<sub>2</sub>, NHR, and NR<sub>2</sub> are extremely poor leaving groups, and the corresponding acid salts of the latter three are similarly difficult to displace (1). The aromatic

tertiary amine functionality may be converted to an acid salt under conditions of high acidity, but the acid salt remains hydrolytically stable (2).

Based upon the physical and chemical properties of amine 3 described above, it must be concluded that it is not subject to hydrolysis, but may form the acid salt of the amine under conditions of high acidity. Therefore, it is concluded that amine 3 should be considered stable when exposed to the conditions of hydrolysis at temperatures and pH levels relevant to environmental and human exposure.

(1) March, J., ed. "Advanced Organic Chemistry", 3rd edition, pp. 312-315, John Wiley & Sons, New York, 1985.

(2) Patai, S., ed. "The Chemistry of the Amino Group", 1st edition, pp.407-498, Interscience Publishers, London, 1968.

**Source** : Unpublished assessment by Dr. Phil Hudnall of the Eastman Chemical Company.

**Reliability** (2) valid with restrictions  
Data are based on analysis of chemical structure.

17.11.2003

### 3.1.3 STABILITY IN SOIL

### 3.2.1 MONITORING DATA

### 3.2.2 FIELD STUDIES

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Type** : Fugacity model level III  
**Media** :  
**Air** : 0.0618 % (Fugacity Model Level III)  
**Water** : 34.1 % (Fugacity Model Level III)  
**Biota** : 0.162 % (Fugacity Model Level II/III)  
**Soil** : 65.7 % (Fugacity Model Level II/III)  
**Method** : other: model calculation  
**Year** : 2003  
**Remark** : Inputs to the program were CAS No. 19248-13-6, a melting point of 0 and a boiling point of 250 degrees C. Emission rates inputted to the program were the model default values of 1000 kg/hour to air, water and soil.  
**Result** : The EPIWIN HENRY Program (v3.10) calculates a Henry's Law Constant of 1.71 E-008 atm-m<sup>3</sup>/mol using the Bond Estimate method.  
**Reliability** : (2) valid with restrictions  
Data were calculated using a model program.  
**Flag** : Critical study for SIDS endpoint

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(3)

### 3.3.2 DISTRIBUTION

### 3.4 MODE OF DEGRADATION IN ACTUAL USE

## 3.5 BIODEGRADATION

|                          |   |   |
|--------------------------|---|---|
| <b>Type</b>              | : | aerobic   |
| <b>Inoculum</b>          | : | other: activated sludge   |
| <b>Concentration</b>     | : | 20 mg/l related to DOC (Dissolved Organic Carbon) related to  |
| <b>Contact time</b>      | : |   |
| <b>Degradation</b>       | : | = 0 (±) % after 28 day(s)   |
| <b>Result</b>            | : | under test conditions no biodegradation observed  |
| <b>Control substance</b> | : | Benzoic acid, sodium salt   |
| <b>Kinetic</b>           | : | %<br>%  |
| <b>Deg. product</b>      | : |   |
| <b>Method</b>            | : | other: OECD Guide-line 301 B and EEC/Annex V C.4  |
| <b>Year</b>              | : | 2003  |
| <b>GLP</b>               | : | yes   |
| <b>Test substance</b>    | : | as prescribed by 1.1 - 1.4  |
| <b>Remark</b>            | : | The positive control yielded < 60 % degradation (59%) by day 14, which did not meet the criteria for a valid test. However, this value was within the normal variability of the Sturm test (+/- 5%), and by the end of the test it had reached 65% biodegradation. The final suspended solids level of the inoculum in the test vessels was 22.7 mg/l, which was less than the maximum recommended (30 mg/l). This may have been a contributing factor in the failure of the positive control to meet the 60% requirement. Since all other criteria for a valid test were met, it was concluded that the test was valid.  |
| <b>Result</b>            | : | At all time points, the percentage of material biodegraded ranged from -3 to 1%. Less CO <sub>2</sub> was evolved from the test vessels than from the blanks at several time points, resulting in negative values. The material was not readily biodegraded under the test conditions.<br><br>The positive control was 59% degraded at day 10 and 65 % degraded at day 28.<br><br>The pH of the BSM at the beginning of the test was 7.489. The pH ranged from 7.450 - 7.894 on day 27. No unusual variation in pH was noted from day 0 to 27. The vessels containing inoculum but no test material released an average of 76.3 mg CO <sub>2</sub> (25.4 mg CO <sub>2</sub> /l) over the test period. The barium hydroxide stock solution needed 48.5 +/- 0.5 ml of titrant compared to 47.9 +/- 0.6 ml for the airline control, indicating that the airline did not contain CO <sub>2</sub> after scrubbing. The average temperature was 22 +/- 0.5 degrees C.   |
| <b>Test condition</b>    | : | Test bacteria: Activated sludge microorganisms were obtained from a domestic wastewater treatment plant. Upon arrival at the test site, the sludge was aerated for approximately 4 hours. A sample of the mixed liquor was homogenized for 2 minutes with a blender, and was allowed to settle for approximately 60 minutes. The supernatant was pipetted off and used in the studies. Numbers of viable microbes in the supernatants (10 <sup>6</sup> E 6 organisms/ml) were estimated using a dip slide.<br><br>Test solutions: Stock solutions of phosphate buffer (8.50 g KH <sub>2</sub> PO <sub>4</sub> , 21.75 g K <sub>2</sub> HPO <sub>4</sub> , 33.4 g Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O, and 0.50 g NH <sub>4</sub> Cl in 1000 ml distilled water), calcium chloride solution (27.50 g CaCl <sub>2</sub> in 1000 ml distilled water) and magnesium sulfate solution (22.50 g MgSO <sub>4</sub> .7H <sub>2</sub> O in 1000 ml distilled water) were made in advance, filter sterilized and refrigerated until needed (up to 6 months). A solution containing 25 mg FeCl <sub>3</sub> .6H <sub>2</sub> O in 100 ml distilled water was made immediately before use. Basal salts medium (BSM) was prepared by mixing 10 ml of the phosphate buffer solution with 800 ml of distilled water, adding 1 ml each of the other solutions, and bringing the volume up to 1 liter. The pH was adjusted to 7.4 +/- 0.2 using 6 N HCl. The inorganic carbon concentration of the BSM was 0.2529 ppm. |

A positive control stock solution of sodium benzoate containing 20 mg DOC/l was prepared (102.9 mg/500 ml purged BSM). The pH of this solution was not adjusted since it was within the required range of 3 - 10.

A stock solution of the test material was not prepared due to the low aqueous solubility of the test material.

Twelve liters of a 0.0125 M solution of barium hydroxide was prepared, filtered, and stored in airtight containers. This material was titrated with 0.05 N HCl (with phenolphthalein as an indicator) when first prepared and at weekly intervals to confirm stability.

Test procedure: A CO<sub>2</sub> scrubbing apparatus was set up to remove CO<sub>2</sub> (at a constant rate) from the air supplied to the test vessels. The air was diverted through a drying column (containing Drierite), a CO<sub>2</sub> absorption column (containing Ascarite II) and flow meters before being bubbled into the test vessels. The estimated rate of air passage through the system was approximately 50-100 ml/min. All test vessels were covered with aluminum foil for the duration of the study. A set of 3 absorber bottles [containing 100 ml of 0.0125 M Ba(OH)<sub>2</sub>] was connected directly to the scrubbed airline and titrated with 0.05 N HCl (with phenolphthalein as an indicator) to assess whether the air supply was free of CO<sub>2</sub>.

BSM (2300 ml) was added to 5 different test vessels, followed by 200 ml of inoculum supernatant. The mixture in each vessel was aerated with CO<sub>2</sub>-free air for approximately 24 hours to purge the system of CO<sub>2</sub>. After the aeration period, test material (27.0 mg/l final concentration; 20 mg DOC/l) was added directly added in small, plastic weigh boats to 2 of the vessels. Purged BSM (500 ml) was then added to these vessels and 2 others that served as negative controls. All five hundred ml of the positive control solution was added to the fifth vessel. Weigh boats were added to the 3 vessels that did not contain test material. Each vessel was agitated with a magnetic stir bar. Three CO<sub>2</sub> absorber bottles were connected in series to the exit airline of each vessel. Each absorber bottle contained 100 ml of 0.0125 M Ba(OH)<sub>2</sub>.

At the beginning of the test, CO<sub>2</sub>-free air was bubbled through the solutions at an estimated rate of 50 – 100 ml/min. At the start of the test and at days 1, 3, 6, 8, 10, 14, 17, 20, 23, and 27, the CO<sub>2</sub> absorber bottles nearest to each vessel were removed for titration with 0.05 N HCl (with phenolphthalein as an indicator). At each time point, the remaining 2 bottles in series were moved one place closer to each vessel, and a new bottle containing 100 ml of fresh 0.0125 M Ba(OH)<sub>2</sub> was placed at the far end of the series. On the 27th day, a 10 ml aliquot of liquid in all three vessels was removed for pH measurement before titration. After titration, concentrated HCl (1 ml) was added to drive off organic carbonate, and the vessels were aerated overnight. Final titrations were performed on day 28. Air temperature was recorded during the test period. DOC measurements were not performed due to the low aqueous solubility of the test material.

Calculations: Lotus 1-2-3 was used for data calculations and tabulations and to generate graphs. The amount (in ml) of titrant used in the blank controls was subtracted from that of test vessels to obtain the true value for the test material. Each 1.0 ml of HCl used corresponded to 1.1 mg of CO<sub>2</sub> produced. The percentage of material biodegraded was calculated as the mg CO<sub>2</sub> produced x 100 / theoretical CO<sub>2</sub> x mg of test material used. The theoretical CO<sub>2</sub> = number of carbon atoms in the test material x MW of CO<sub>2</sub> / MW of the test material. The CO<sub>2</sub> concentrations were calculated to the nearest 0.1 mg/l, and the biodegradation values were rounded up to the nearest whole percent.

Criteria for a valid test: The criteria for a valid test were as follows: 1)

- positive control reached  $\geq 60\%$  biodegradation by day 14, 2) the difference of extremes of replicates were less than 20% at the plateau, end of the test or at day 10 (as appropriate), 3) The inorganic carbon content of the test material in the BSM at the beginning of the test was  $< 5\%$  of the total carbon content, 4) the total CO<sub>2</sub> evolution in the blank was not  $> 40$  mg CO<sub>2</sub>/l, and 5) the CO<sub>2</sub> was scrubbed from the incoming airline.
- Test substance** : Purity of the material was determined to be 97.8 % (weight percent) by gas chromatography with flame ionization detection. The structure was confirmed by gas chromatography with mass spectrometric detection.
- Reliability** : (1) valid without restriction  
The test is a guideline study, which met all criteria for a valid test except 1.
- Flag** : Critical study for SIDS endpoint

(12)

**3.6 BOD5, COD OR BOD5/COD RATIO****3.7 BIOACCUMULATION****3.8 ADDITIONAL REMARKS****4.1 ACUTE/PROLONGED TOXICITY TO FISH**

- Type** : static
- Species** : Pimephales promelas (Fish, fresh water)
- Exposure period** : 96 hour(s)
- Unit** : mg/l
- NOEC** : = 5.08 measured/nominal
- LC50** : = 7.4 measured/nominal
- LC100** : = 10.8 measured/nominal
- Limit test** : no
- Analytical monitoring** : yes
- Method** : other: OECD: TG-203 and EEC/Annex V C.1.
- Year** : 2003
- GLP** : yes
- Test substance** : as prescribed by 1.1 - 1.4
- Result** : None of the control fish or fish exposed to concentrations  $\leq 5.08$  mg/l died or had abnormal behavior during the study. The mortality rate of fish exposed to 10.8 mg/l was 86% (5/7) by 24 hours. The two remaining fish exposed to this concentration had depressed behavior at 24 hours. All fish exposed to 10.8 died after 48 hours of exposure. The 24 hour LC50 value was 7.9 mg/l. The 48, 72 and 96 hour LC50 values were 7.4 mg/l. The highest concentration causing no ( $\leq 10\%$ ) mortality and the no observable effect level was 5.08 mg/l. The lowest concentration causing 100% mortality was 10.8 mg/l.

Throughout the study, the temperatures of the solutions were maintained at 20 degrees C. Dissolved oxygen concentrations of the control and test solutions ranged from 9.6 - 7.4 mg/l. The pH values of the control and test solutions ranged from 8.0 - 8.3 and 8.0 - 8.6, respectively. The temperature, pH values and dissolved oxygen concentrations were considered to be acceptable.

Throughout the study, all solutions appeared clear and colorless. The nominal concentrations were 0.625, 1.25, 2.5, 5 and 10 mg/l. Corresponding analytical concentrations were 0.74, 1.07, 2.15, 5.08 and

**Test condition**

10.8 mg/l, respectively. Analytical concentrations were 4.5 % lower to 17.1% higher than nominal.

: Organisms: Juvenile fathead minnows were cultured in 200 L stainless steel tanks provided with a continuous flow of filtered, treated, tempered water. The tanks were continuously aerated by passing oil-free filtered air through air stones. The tanks were maintained at 20 degrees C and illuminated with fluorescent lighting for 16 hours followed by a 30 min transition period leading to 8 hours of darkness. The fish were fed with commercial fish food with or without young live Daphnia. The diets were analyzed routinely for contaminants. No known contaminants that could interfere with the outcome of the test were identified. Behavioral observations were made daily.

Fish were acclimated to test water for at least two weeks prior to testing. They were not fed for 24 hours prior to exposure. They were randomized to 14 sets of 7 fish each. Two sets of minnows (7/set) were killed before the start of the test to determine average wet weight (0.26 and 0.20 g/set) and mean standard length (2.5 and 2.4 cm/set). Fish were placed randomly (no more than 3-4 at a time) into each of two sets of test and control vessels (7 per vessel). Biological loading was kept below 1.0 g wet weight per liter of solution.

Test water: The water was pumped from Lake Ontario, treated by a water treatment facility, and stored in a large underground storage vessel. Water from this vessel was subsequently pumped into the laboratory where it passed through polypropylene filters, activated carbon filter tubes, and another set of polypropylene filters. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then tempered to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Average values for hardness and total alkalinity (both as CaCO<sub>3</sub>) were 123.5 and 90.8 mg/l, respectively. The quality of the water was monitored twice per year. The water contained no contaminants at concentrations that would interfere with the outcome of the study.

Test material: Exposure solutions containing the test material at nominal concentrations of 0 (control), 0.625, 1.25, 2.5, 5 and 10 mg/l were prepared by directly adding the appropriate amounts of test material (0, 0.0125, 0.025, 0.05, 0.1 and 0.2 g) to separate test vessels (Pyrex cuboidal glass tanks) containing 20 liters of dilution water. The approximate headspace was 2700 cm<sup>3</sup>. The solutions were stirred for an hour with a stir bar on a stir plate. The solutions settled for 15 - 20 minutes before measurement of physical parameters.

Test conduct: The test was performed as a 96-hour static exposure. Fish were added directly to the test solutions, which were prepared in duplicate. Test solutions were maintained at 20 +/- 1 degrees C, and illuminated as previously described. Test chambers were covered during the study, and were not aerated. Fish were not fed.

Animals were observed for mortality and signs of stress at 0, 4, 24, 48, 72 and 96 hours. Temperature, dissolved oxygen concentration and pH of the fresh and seasoned exposure solutions were measured at the beginning and end of each 24-hour period. The appearances of the exposure solutions at 0, 4, 24, 48, 72 and 96 hours were noted. Samples of the exposure solutions were collected at the start and end of the test (or when complete mortality was observed) and analyzed for concentration of test material using gas chromatography with flame ionization detection.

Analysis of data: The concentrations of material in the solutions were determined by calculating the average of the concentrations of solutions collected from individual replicates at the beginning and end of each

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exposure. Statistical analyses were performed using TOXSTAT statistical software. The LC50 values at 24, 48, 72 and 96 hours were calculated using the Trimmed Spearman-Karber method.

- The test was considered valid if control mortality was  $\leq 10\%$ , dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was  $20 \pm 1$  degrees C, the pH did not vary by more than one unit and there were no abnormal occurrences that could influence the outcome.
- Test substance** : Purity of the material was determined to be 97.8 % (weight percent) by gas chromatography with flame ionization detection. The structure was confirmed by gas chromatography with mass spectrometric detection.
- Reliability** : (1) valid without restriction  
The study was performed according to the guideline. There were no deviations that would affect the outcome of the test.
- Flag** : Critical study for SIDS endpoint
- 22.09.2003 (10)

### 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

- Type** : static
- Species** : Daphnia magna (Crustacea)
- Exposure period** : 48 hour(s)
- Unit** : mg/l
- NOEC** : = 1.14 measured/nominal
- EC50** : = 4.8 measured/nominal
- EC100** : = 10.72 measured/nominal
- Limit Test** : no
- Analytical monitoring** : yes
- Method** : other: OECD: TG-202 and EEC/Annex V C.2
- Year** : 2003
- GLP** : yes
- Test substance** : as prescribed by 1.1 - 1.4
- Result** : None of the control daphnids or daphnids exposed to 0.72 or 1.14 mg/l were immobile or had abnormal behavior during the study. None of the daphnids exposed to 2.26 mg/l were immobile after 24 hours. However, 15% immobility was observed in this group at 48 hours. After 24 and 48 hours, immobility was noted in 5% and 40% of daphnids exposed to 5.24 mg/l, and 70% and 100% of daphnids exposed to 10.72 mg/l. The 24 and 48 hour EC50 values were 9.0 and 4.8 mg/l, respectively. The highest concentration causing no ( $\leq 10\%$ ) immobility was 1.14 mg/l. The lowest concentration causing 100% immobility was 10.72 mg/l.
- Throughout the study, the temperatures of the solutions were maintained at 20 degrees C and the pH values ranged from 8.2 to 8.6. Dissolved oxygen concentrations of the control and test solutions ranged from 8.6 - 9.6 mg/l and 8.5 - 9.6 mg/l, respectively. The temperature, pH values and dissolved oxygen concentrations were considered to be acceptable.
- Throughout the study, all solutions appeared clear and colorless. The nominal concentrations were 0.625, 1.25, 2.5, 5 and 10 mg/l. Corresponding analytical concentrations were 0.72, 1.14, 2.26, 5.24 and 10.72 mg/l, respectively. Analytical concentrations were 2.9 % lower to 28.4% higher than nominal.
- Test condition** : Organisms: Adult Daphnia magna were reared within the testing facility in 100-l stainless steel tanks supplied continuously with filtered, treated, tempered (20 degrees C) water. The tanks were continuously aerated by passing oil-free filtered air through air stones. The facility was illuminated with fluorescent lighting for 16 hours, followed by a 30 minute transition period leading to 8 hours of darkness. The daphnia were fed a spinach-fish food slurry with or without a yeast-Cerophyll leaves- trout chow mixture

or green algae. The diets were analyzed routinely for contaminants. Contaminant concentrations were lower than those that could interfere with the outcome of the test. Daphnia were observed daily for feeding and other behaviors.

**Test water:** The water was pumped from Lake Ontario, treated by a water treatment facility, and stored in a large underground storage vessel. Water from this vessel was subsequently pumped into the laboratory where it passed through polypropylene filters, activated carbon filter tubes, and another set of polypropylene filters. The filtered water stream was treated with sodium thiosulfate to further reduce trace levels of residual chlorine. The water was then tempered to 20 +/- 2 degrees C and distributed to an open basin for seasoning prior to use. Average values for hardness and total alkalinity (both as CaCO<sub>3</sub>) were 123.5 and 90.8 mg/l, respectively. The quality of the water was monitored twice per year. The water contained no contaminants at concentrations that would interfere with the outcome of the study.

**Test material:** Exposure solutions containing the test material at nominal concentrations of 0 (control), 0.625, 1.25, 2.5, 5 and 10 mg/l were prepared by directly adding the appropriate amounts of test material (0, 0.0125, 0.025, 0.05, 0.1 and 0.2 g) to separate vessels containing 20 liters of dilution water. The solutions were stirred for an hour with a stir bar on a stir plate. The solutions settled for 15 - 20 minutes before measurement of physical parameters. Aliquots of the solutions (200 ml) were removed from the middle of the water column and transferred to test vessels (250 ml Pyrex glass beakers). Headspace was 80 cm<sup>3</sup>.

**Test conduct:** Approximately 24 hours before the start of the test, gravid adult daphnids were transferred into 20 cm diameter bowls containing test water and fed. The neonates produced in the following 24 hour period were collected by pipette and transferred directly into duplicate test vessels (10/vessel). No more than 5 organisms were transferred into each vessel at a time. The test was performed as a 48-hour static exposure. Test solutions were maintained at 20 +/- 1 degrees C, and illuminated as previously described. Test chambers were covered during the study, and were not aerated. Daphnia were not fed during the test.

Animals were observed for mobility and signs of stress at 0, 4, 24, and 48 hours. Temperature, dissolved oxygen concentration and pH of the solutions were measured at the start and end of the experiment, or when complete immobility occurred. The appearances of the exposure solutions at 0, 4, 24 and 48 hours were noted. Samples of the exposure solutions were collected at the start and end of the study (or when complete immobility was observed) and analyzed for concentration of test material using gas chromatography with flame ionization detection.

**Analysis of data:** The concentrations of material in the solutions were determined by calculating the geometric mean of the concentration in each replicate at the beginning and end of each exposure. Concentrations in each replicate were then averaged. Statistical analyses were performed using TOXSTAT statistical software. The EC<sub>50</sub> values (concentrations causing immobility in 50% of the animals) at 24 and 48 hours were calculated using the Probit method.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 2 mg/l, the temperature was 20 +/- 1 degrees C, the pH did not vary by more than 1.5 units, control daphnids were not trapped at the surface and there were no abnormal occurrences that could influence the outcome.

**Test substance** : Purity of the material was determined to be 97.8 % (weight percent) by gas chromatography with flame ionization detection. The structure was

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**Reliability** : confirmed by gas chromatography with mass spectrometric detection.  
: (1) valid without restriction  
The study was performed according to the guideline. There were no deviations that would affect the outcome of the test.

**Flag** : Critical study for SIDS endpoint  
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### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

**Species** : Selenastrum capricornutum (Algae)  
**Endpoint** : other: biomass and growth rate  
**Exposure period** : 72 hour(s)  
**Unit** : mg/l  
**NOEC** : = .56  
**EbC50** : = 1.95  
**ErC50** : = 4.78  
**Limit test** : no  
**Analytical monitoring** : yes  
**Method** : OECD Guide-line 201 "Algae, Growth Inhibition Test"  
**Year** : 2003  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4  
**Result** : Exposure to 0.56 mg/l had no significant effect on biomass or growth rate at any time point. At 72 hours (but not at earlier time points), 1.18 mg/l had an inhibitory effect on growth rate (-5%) and biomass (- 15%). At 24 hours and later time points, a dose-dependent inhibition of biomass and growth rate was noted at concentrations > = 2.32 mg/l. The EbC50 (0-72 hour) value was 1.95 mg/l, and the ErC50 (0-72 hour) value was 4.78 mg/l.

The average nominal concentrations of material in the test flasks at the beginning of the test were 0.625, 1.25, 2.5, 5.0 and 10.0 mg/l. Corresponding analytical concentrations were 0.56, 1.18, 2.32, 4.72 and 9.91 mg/l. The amount of material lost over the course of the experiment ranged from 0 - 17.4%. The analytical concentration that lost 17.4% loss of the material was 1.18 mg/l. All other solutions lost from 0 - 6.3% of the material. According to the authors, the test material was stable under the test conditions.

Results of the photostability tests were similar to those in flasks containing test material and algae. The control solutions that were exposed to light or were in the dark exhibited a 4.38% gain and 2.09% loss of test material, respectively.

The mean temperature and illumination were 24 degrees C and 743.3 foot-candles (range 741 - 745 foot-candles) throughout the test. The pH of test and control solutions ranged from 7.52 - 8.18. By the end of the test, the pH values of the test solutions had not deviated by more than 1.5 units (as required by the guideline). The shaker speed was maintained at 100 rpm.

The test was considered to be valid since the mean cell concentration in control cultures increased by a factor of 104-fold within 72 hours. The control cells exhibited normal log growth.

**Test condition** : Test Organisms: A 4-day culture of Selenastrum capricornutum (passage 2 in liquid algal medium) was used as the test algae. Several passages were performed prior to the test to confirm exponential growth.

Test medium: Sterile growth medium was prepared using high quality distilled water. The pH of the medium was measured and adjusted to 7.5 (+/- 0.1) using NaOH.

Test material stock solution: Test material (0.0130 g) was added to 130.93 g of algal growth medium (to produce a nominal concentration of 99.28 mg/l). The solution was sonicated for approximately 10 minutes to fully dissolve the material, and was filtered through a 0.45 micron membrane filter. Serial dilutions were performed to produce solutions containing 10.0, 5.0, 2.5, 1.25 and 0.625 mg/l. Aliquots of each solution were removed for analysis of concentration at time 0.

Test conduct: All steps were carried out aseptically in a hood to prevent contamination. Test vessels were sterile, conditioned 250 ml Erlenmeyer flasks. Each test material stock solution (100 ml) was added to 5 flasks and test medium that did not contain test material was added to 3 flasks. Algae (324 microliters of algal stock culture to achieve an initial cell density of  $1 \times 10^4$  cells/ml) were added to 3/5 flasks that contained the highest concentration of test material and all other flasks. The two flasks that contained the highest concentration test material without algae served as photostability controls. One of the flasks was exposed to light and one was wrapped in foil to shield it from light. All flasks were secured with foam stoppers and transferred to a shaking incubator (24 degrees C, 100 rpm). They were illuminated at an average of 743.3 +/- 1.7 footcandles throughout the study. Flasks were rotated randomly at 24-hour intervals after cells were counted.

Temperature, light intensity, and shaker speed (rpm) were assessed at 0, 24, 48, and 72 hours. Concentrations of test material in the flasks that contained algae also were assessed at these times. The pH was assessed at time 0 and after 72 hours. Concentrations of test material in the photostability controls also were measured at 0 and 72 hours. Concentrations of test material were analyzed using gas chromatography with flame ionization detection (GC/FID). The exposure concentration was calculated as the geometric mean of the test concentrations analyzed at the 4 time points.

Cell counts were performed after 24, 48 and 72 hours of exposure using a calibrated Coulter Counter. Flasks were swirled to achieve a uniform cell suspension and 4.0 ml were removed for counting. The mean algal cell count for the test and control curves was calculated. Two measures of growth [biomass (area under the growth curve) and growth rate] were used to determine the effect of the material on algae. The percentage inhibitions of biomass and growth rate were calculated for each concentration and plots of concentration vs. percentage inhibition of biomass and growth rate were made. The concentrations that produced a 50% inhibition of growth (biomass, EbC50) and growth rate (ErC50) relative to control were calculated by fitting nonlinear regression models to the data.

The test was considered valid if the mean cell concentration in the control cultures increased by a factor of at least 16 within 72 hours.

- Test substance** : Purity of the material was determined to be 97.8 % (weight percent) by gas chromatography with flame ionization detection. The structure was confirmed by gas chromatography with mass spectrometric detection.
- Reliability** : (1) valid without restriction  
The study was performed according to the guideline. There were no deviations that would affect the outcome of the test.
- Flag** : Critical study for SIDS endpoint  
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#### 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

## 4.5.1 CHRONIC TOXICITY TO FISH

## 4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

## 4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

## 4.6.2 TOXICITY TO TERRESTRIAL PLANTS

## 4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

## 4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

## 4.7 BIOLOGICAL EFFECTS MONITORING

## 4.8 BIOTRANSFORMATION AND KINETICS

## 4.9 ADDITIONAL REMARKS

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

## 5.1.1 ACUTE ORAL TOXICITY

|                          |   |
|--------------------------|---|
| <b>Type</b>              | : LD50  |
| <b>Value</b>             | : = 400 mg/kg bw  |
| <b>Species</b>           | : rat   |
| <b>Strain</b>            | : other: not listed   |
| <b>Sex</b>               | : no data   |
| <b>Number of animals</b> | : 6   |
| <b>Vehicle</b>           | :   |
| <b>Doses</b>             | : 200 - 800 mg/kg   |
| <b>Method</b>            | : other   |
| <b>Year</b>              | : 1964  |
| <b>GLP</b>               | : no  |
| <b>Test substance</b>    | : as prescribed by 1.1 - 1.4  |
| <b>Remark</b>            | : This is a supporting study for the SIDS endpoint.   |
| <b>Result</b>            | : The LD50 value as 400 mg/kg. Animals died within 1/2 hour to 1 day of treatment. Autopsy results were negative. Symptoms of toxicity were weakness, tremors and rapid respiration. Survivors gained weight over 14 days.    |
| <b>Test condition</b>    | : Six rats were administered 200 to 800 mg/kg/ test material orally (presumably by gavage). Mortality and symptoms of toxicity were monitored over 14 days. Animals were weighed at the beginning of the study and on day 14. |
| <b>Test substance</b>    | : Purity of the test material is unknown.   |
| <b>Reliability</b>       | : (2) valid with restrictions   |

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Basic data are given. The number of animals receiving each dose, the number of animals that died at each dose and the method used to calculate the LD50 value were not mentioned.

(2)

**Type** : LD50  
**Value** : = 400 - 800 mg/kg bw  
**Species** : mouse  
**Strain** : other: not listed  
**Sex** : no data  
**Number of animals** : 10  
**Vehicle** :  
**Doses** : 200 - 3200 mg/kg  
**Method** : other  
**Year** : 1964  
**GLP** : no  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : This is a supporting study for the SIDS endpoint. Judging from the way other studies have been performed in this laboratory, it is likely that 2 animals/group were given 200, 400, 800, 1600 and 3200 mg/kg/ test material.

**Result** : The LD50 value as 400 - 800 mg/kg. Animals died within 1 hour to 1 day of treatment. Symptoms of toxicity were weakness, severe ataxia, tremors and convulsions. Survivors gained weight over 14 days.

**Test condition** : Ten mice were administered 200 to 3200 mg/kg/ test material orally (presumably by gavage). Mortality and symptoms of toxicity were monitored over 14 days. Animals were weighed at the beginning of the study and on day 14.

**Test substance** : Purity of the test material is unknown.  
**Reliability** : (2) valid with restrictions  
Basic data are given. The number of animals receiving each dose, the number of animals that died at each dose and the method used to calculate the LD50 value was not mentioned.

(2)

### 5.1.2 ACUTE INHALATION TOXICITY

**Type** : LC50  
**Value** : > .238 mg/l  
**Species** : Rat  
**Strain** : other: not listed  
**Sex** : no data  
**Number of animals** : 3  
**Vehicle** :  
**Doses** : 0.238 mg/l (37.79 ppm)  
**Exposure time** : 6 hour(s)  
**Method** : other  
**Year** : 1964  
**GLP** : No  
**Test substance** : as prescribed by 1.1 - 1.4  
**Result** : None of the animals died. Roughing of hair and vasodilation (extremities were pink) were observed after 5 or 20 minutes of exposure, respectively. Facial muscle fibrillation was noted after 25 minutes. Animals gained weight over the 2 week observation period.

**Test condition** : Animals were administered 0.238 mg/l (37.79 ppm) test material for 6 hours by inhalation. The test material was administered at a rate of 3.5 l/min through a gas washing bottle that was maintained at room temperature. The chamber temperature was 26 degrees C. Mortality and symptoms of toxicity were monitored over a 14 day period. Animals were weighed at the beginning and end of the study.

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|                                   |   |     |
|-----------------------------------|---|-----|
| <b>Test substance Reliability</b> | : Purity of the test material is unknown.<br>: (2) valid with restrictions<br>Basic data are given. Only 3 animals were tested.   | (2) |
| <b>Type</b>                       | : LC50  |     |
| <b>Value</b>                      | : > 4.58 mg/l   |     |
| <b>Species</b>                    | : Rat   |     |
| <b>Strain</b>                     | : other: not listed   |     |
| <b>Sex</b>                        | : no data   |     |
| <b>Number of animals</b>          | : 3   |     |
| <b>Vehicle</b>                    | :   |     |
| <b>Doses</b>                      | : 4.58 mg/l (727.3 ppm)   |     |
| <b>Exposure time</b>              | : 6 hour(s)   |     |
| <b>Method</b>                     | : other   |     |
| <b>Year</b>                       | : 1964  |     |
| <b>GLP</b>                        | : No  |     |
| <b>Test substance Result</b>      | : as prescribed by 1.1 - 1.4<br>: None of the animals died. Roughing of hair, blinking and accelerated respiration were observed after 5 minutes of exposure. Lacrimation, nasal discharge and salivation were noted after 15 minutes. Vasodilation (extremities were pink) occurred after 4 hours, and tremors of the head after 4.5 hours. Gross tremors of the trunk were noted after 5.5 hours of exposure. Animals gained weight over the 2 week observation period. |     |
| <b>Test condition</b>             | : Animals were administered 4.58 mg/l (727.3 ppm) test material vapor for 6 hours by inhalation. The test material was administered at a rate of 2 l/min through a gas washing bottle that was maintained at 100 degrees C. The chamber temperature was 27 degrees C. Mortality and symptoms of toxicity were monitored over a 14 day period. Animals were weighed at the beginning and end of the study.   |     |
| <b>Test substance Reliability</b> | : Purity of the test material is unknown.<br>: (2) valid with restrictions<br>Basic data are given. Only 3 animals were tested.   | (2) |

### 5.1.3 ACUTE DERMAL TOXICITY

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

### 5.2.1 SKIN IRRITATION

### 5.2.2 EYE IRRITATION

### 5.3 SENSITIZATION

### 5.4 REPEATED DOSE TOXICITY

### 5.5 GENETIC TOXICITY 'IN VITRO'

**Type** : Bacterial reverse mutation assay

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**System of testing** : Salmonella typhimurium/TA98, 100, 1535, 1537, and Escherichia coli/WP2uvrA(pKM101)  
**Test concentration** : 10.0, 33.3, 100, 333, 1000, 3330 and 5000 micrograms/plate (S. typhimurium); 33.3, 100, 333, 1000, 3330 and 5000 micrograms/plate (E. coli)  
**Cytotoxic concentr.** : > = 1000 micrograms/plate  
**Metabolic activation** : with and without  
**Result** : negative  
**Method** : OECD Guide-line 471  
**Year** : 2002  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4  
**Remark** : This is the critical study for the mutagenesis endpoint.  
**Result** : In the first mutagenicity test, no positive increases were observed in the mean number of revertants per plate with any of the Salmonella strains incubated with test material in either the presence or absence of S9 mix and with E. coli WP2uvrA(pKM101) in the absence of S9 mix. Cytotoxicity was observed in the Salmonella strains at concentrations of 1000 to 5000 micrograms/plate in the absence of S9 mix and in strain TA100 at 3330 micrograms/plate in the presence of S9 mix. Cytotoxicity was observed in E. coli strain WP2uvrA at > = 1000 micrograms/plate in the absence of S9 mix. Cultures of E. coli strain WP2uvrA in the presence of S9 were not evaluated due to problems with the controls (see test conditions). No precipitate was observed in any of the plates.

As noted in the test conditions section, the test with E. coli strain WP2uvrA in the presence of S9 had to be performed three times in order to achieve a valid result. In the second test with E. coli strain WP2uvrA and S9 mix, the mean number of revertants in the vehicle control (32/plate) was not within the acceptable range for this strain (80-350 revertants/plate). For this reason, a third experiment with E. coli strain WP2uvrA in the presence of S9 mix was conducted. In this experiment, all data were acceptable and no positive increases in the number of revertants per plate were observed in cells treated with test material in the presence of S9 mix. Cytotoxicity was observed in E. coli strain WP2uvrA at > = 3330 micrograms/plate in the presence of S9 mix.

In the confirmatory mutagenicity test, all data were acceptable and no positive increases in the number of revertants per plate were observed in any of the bacteria incubated with test material (in the absence or presence of S9 mix). Cytotoxicity was noted in all Salmonella strains incubated without S9 mix and test material concentrations > = 3330 micrograms/plate. Cytotoxicity also was observed in Salmonella strains TA98, TA100 and E. coli strain WP2uvrA incubated with 3330 or 5000 micrograms/plate with S9. No precipitate was observed in any of the plates.

The initial tests with all strains [except E. coli WP2uvrA(pKM101) in the presence of S-9], the third initial test with E. coli WP2uvrA(pKM101) in the presence of S-9, and the confirmatory tests with all strains were valid, since they met all criteria for a valid study.

**Test condition** : Test strains: The S. typhimurium and E. coli strains were obtained from Dr. Bruce Ames, University of California Berkeley and the National Collection of Industrial Bacteria, Torry Research Station, Scotland, respectively. Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 ml/ml culture) and freezing small aliquots at -60 to -80 degrees C. Master plates were prepared by streaking each test strain from a frozen permanent stock onto minimal agar supplemented with histidine, biotin, ampicillin and/or tryptophan (depending on the strain). Tester strain master plates were stored at > 0 to 10 degrees C. Overnight cultures were inoculated by transferring colonies from the master plates to flasks containing culture medium. Inoculated flasks were placed in a shaker/incubator (125 +/- 25 rpm, 37 +/- 2 degrees C). Cultures in late log

phase were harvested once a predetermined turbidity was reached (at least  $0.5 \times 10^9$  cells/ml). Test stains were checked for rfa wall mutation (all Salmonella strains), pKM101 plasmid (Salmonella TA98 and TA100 and E. coli only), and characteristic number of spontaneous revertants (all strains) on the day the mutagenicity test was conducted.

**Test medium:** The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution supplemented with 2.5% Oxoid Nutrient Broth No. 2. Bottom agar was Vogel-Bonner minimal medium E supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Overlay agar contained 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with either 10 ml of 0.5 mM histidine/biotin solution per 100 ml agar or 0.5 mM tryptophan solution per 100 ml agar.

**S-9 mix:** S9 homogenate was purchased from Molecular Toxicology Inc. This was prepared from male Sprague-Dawley rats that had been injected i.p. with 500 mg/kg Aroclor 1254. S-9 mix was prepared immediately prior to use.

**Concentrations of test material:** The test material was insoluble in water at 100 mg/ml. The most concentrated stock solution prepared was 100 mg per ml of DMSO. The test material remained in solution at this concentration and all succeeding dilutions that were prepared. The concentrations tested in both tests (10.0, 33.3, 100, 333, 1000, 3330 and 5000 micrograms/plate for Salmonella with or without S-9; and 33.3, 100, 333, 1000, 3330 and 5000 micrograms/plate for E. coli with or without S9) were selected based on the results of a dose range-finding study using test strains TA100 and WP2uvrA(pKM101) and 10 concentrations of test material from 6.67 to 5000 micrograms/plate (both in the presence and absence of S-9 mix).

**Positive, negative and sterility controls:** Positive controls [2-aminoanthracene (2.5 and 5.0 micrograms/plate), 2-nitrofluorene (1.0 micrograms/plate), sodium azide (2.0 micrograms/plate), ICR-191 (2.0 micrograms/plate), and 4-nitroquinoline-N-oxide (2.0 micrograms/plate)] were run concurrently. DMSO (50 microliters) was used as a vehicle control. The most concentrated test material dilution (50 microliters) and S-9 mix (500 microliters) were tested for sterility by plating on selective agar.

**Test conduct:** A plate incorporation methodology was used. Test material or positive control (50 microliters), test strains (100 microliters) and S-9 mix or vehicle (500 microliters) were combined in 2.0 ml of molten, selective top agar maintained at  $45 \pm 2$  degrees C. This was overlaid onto 25 ml of minimal agar that had been plated into 15 x 100 mm Petri dishes. All concentrations of test material, vehicle controls and positive controls were plated in triplicate. Revertant colonies were counted after  $52 \pm 4$  hours of inverted incubation at  $37 \pm 2$  degrees C. The condition of the background lawn was evaluated for evidence of cytotoxicity and precipitate. The full complement of bacterial strains was tested in two separate experiments.

Since the bacterial background lawn in the vehicle-treated WP2uvrA(pkM101) cells in the presence of S9 was reduced and no revertants were observed in the first test, the treated WP2uvrA(pkM101) cells were not scored and the first experiment with WP2uvrA(pkM101) was repeated. In the second test, the mean number of revertants in the vehicle control (32/plate) was not within the acceptable range for this strain (80-350 revertants/plate). Therefore, an additional test was performed. The results of the third test were valid.

**Evaluation:** The numbers of revertant colonies were counted with an automatic colony counter or by hand. The mean number of revertants and standard deviation were calculated. Various criteria were established to

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constitute a valid assay (test strain integrity, characteristic number of spontaneous revertants, cell density  $> = 0.5 \times 10^9$ , at least a 3-fold increase in revertants in positive controls, and a minimum of 3 non-toxic doses). A positive response was indicated by at least a 2 or 3 fold increase in mean revertant number (depending on the bacterial tester strain).

**Test substance** : Purity of the test material was not confirmed in this study. However, the lot of test material used (062702) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 97.8 %.

**Conclusion** : Material was not genotoxic under conditions of this assay.

**Reliability** : (1) valid without restriction  
This was a well-documented guideline study.

(11)

**Type** : Chromosomal aberration test  
**System of testing** : Chinese Hamster Ovary (CHO) Cells  
**Test concentration** :  
**Cytotoxic concentr.** :  
**Metabolic activation** : with and without  
**Result** : negative  
**Method** : OECD Guide-line 473  
**Year** : 2003  
**GLP** : yes  
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : This is the critical study for the chromosomal aberration endpoint.  
**Result** : Without activation: In the initial study without metabolic activation, no dividing cells or cell monolayers were observed in cultures treated with 882, 1260 or 1800 micrograms/ml. Unhealthy cell monolayers and debris were observed in cultures treated with 617 micrograms/ml. A slight precipitate was noted in cells treated with 420 micrograms/ml. Reductions of 0%, 0%, 0%, 4%, 84% and 87% were observed in the mitotic indices of the cultures treated with 148, 211, 302, 432, 617 and 882 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 148, 211, 302 and 432 micrograms/ml. The cultures treated with 432 micrograms/ml had approximately a 45% reduction in confluence, indicating that this was a valid high dose for analysis.

In the confirmatory study without activation, unhealthy monolayers, dead cells, and no dividing cells were found in cells treated with concentrations  $> = 300$  micrograms/ml. A severe reduction in dividing cells was noted at 200 micrograms/ml. Reductions of 0%, 0%, 46%, 39%, 100%, 100%, 100%, 100% and 100% were observed in the mitotic indices of cultures treated with 12.5, 25.0, 50.0, 100, 200, 300, 400, 500 and 600 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 12.5, 25.0, 50.0, and 100 micrograms/ml.

No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures from either study.

With activation: In the initial study with metabolic activation, toxicity was noted at concentrations  $> = 882$  micrograms/ml. Reductions of 0%, 0%, 0%, 0%, 0% and 56% were observed in the mitotic indices of cultures treated with 148, 211, 302, 432 and 617 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 211, 302, 432 and 617 micrograms/ml. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures.

In the confirmatory study with metabolic activation, toxicity was noted in cells treated with  $> = 500$  micrograms/ml. Reductions of 2%, 5%, 0% and 58% were observed in the mitotic indices of cultures treated with 100, 200, 300 and 400 micrograms/ml, respectively. Chromosomal aberrations were

**Test condition**

analyzed from cultures treated with 100, 200, 300 and 400 micrograms/ml. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures from this study.

All criteria for validity were met in each study (with the exception that in the tests without metabolic activation, the highest concentration analyzed did not cause at least a 50% reduction in mitotic index). Higher concentrations were not evaluated due to excessive toxicity.

: Cells: The Chinese hamster ovary cells used in the assay (CHO-WBL) were from a permanent cell line originally obtained from Dr. S. Wolff, University of California, San Francisco. Stock cultures were maintained for up to 8 weeks after thawing. Mycoplasma testing was performed twice during this period. Cells were grown at 37 +/- 2 degrees C (in 5% +/- 1.5% CO2 in air) in McCoy's 5a culture medium which was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin G and 100 micrograms/ml streptomycin.

S9 mix: S9 was isolated from the liver of rats (sex not stated) 5 days after i.p. treatment with 500 mg/kg Aroclor 1254. S9 was stored frozen at < = -60 degrees C until use. S9 mix was prepared by adding an energy-producing system (1.8 mM NADP plus 10.5 mM isocitric acid) to S9 (1.5%).

Test material and negative and positive controls: The test material was immiscible in water. In DMSO, solutions of 200 and 399 mg/ml appeared transparent and light yellow in color. A solution of 100 mg/ml was transparent and colorless. These solutions (10 microliters/ml) were tested for solubility in culture medium. Concentrations of 2000 and 3990 micrograms/ml precipitated in the medium and increased the pH to 9.5 - 10.0. At 1000 micrograms/ml, the precipitate went back into solution with slight agitation and the pH was 9.0. The highest concentration to be used in the tests was 1800 micrograms/ml, which was slightly greater than the OECD-recommended high dose for the test (10 mM). In each test, the primary stock and its dilutions were dosed at 1% v/v (10.0 micrograms/ml). The solvent control was 10 microliters/ml DMSO. The positive controls were 0.2 - 1.5 micrograms/ml mitomycin C (0.75 and 1.5 micrograms/ml in the initial test without activation and 0.2 and 0.4 micrograms/ml in the confirmatory test without activation) and 5.0 and 10.0 micrograms/ml cyclophosphamide (in both tests with activation). Both positive controls were dissolved in water.

Initial test: Cultures were initiated by seeding approximately 0.9 x 10E6 cells per 75 cm2 flask into a total of 10 ml of complete McCoy's 5a medium. For the test without metabolic activation, one day after culture initiation, cultures were incubated with test material or the negative or positive control for 3.0 hrs at 37 +/- 2 degrees C. For the test with metabolic activation, one day after culture initiation, cells were incubated for approximately 3.0 hours with test material or the negative or positive control and S9 mix in McCoy's 5a medium that did not contain fetal bovine serum. Replicate cultures for each concentration of test material (12.3, 17.5, 25.0, 35.7, 51.0, 72.8, 104, 148, 211, 302, 432, 617, 882, 1260 and 1800 micrograms/ml), positive control, vehicle and untreated controls were prepared. Cultures with or without S9 were then washed with buffered saline, and incubated with complete McCoy's 5a medium for 17 hours. Colcemid (0.1 micrograms/ml) was present during the last 2 +/- 0.5 hours of incubation. Cells were visually inspected for cytotoxicity prior to harvest. Cells were then trypsinized and spun in a centrifuge. The supernatant was discarded and the cells were swollen with 75 mM KCl hypotonic solution. The cells were then fixed with an absolute methanol: glacial acetic acid (3:1, v:v) fixative. They were then placed on glass slides and air-dried. Cells were stained with 5% Giemsa and analyzed for mitotic index and chromosomal aberrations.

Confirmatory assay: The test with metabolic activation was conducted the same as in the initial test, but with different concentrations of test material (100, 200, 300, 400, 500, 600 and 800 micrograms/ml). In the test without metabolic activation, the test material (6.25, 12.5, 25.0, 50.0, 100, 200, 300, 400, 500 and 600 micrograms/ml), positive control and negative controls were incubated with the cells for 19.7 hours (instead of 3). For both tests, Colcemid was present for the last 2.0 +/- 0.5 hours of incubation. The slides were prepared as described for the previous test.

Evaluation: Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 21 +/-2 were analyzed. One hundred cells (if possible) were analyzed from each replicate of the vehicle control, 4 concentrations of the test material, and one concentration of positive control for the different types of chromosomal aberrations. At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations. The number of mitotic cells in 1000 cells was determined and the ratio expressed as the percentage of mitotic cells. Percent polyploidy and endoreduplication were analyzed by evaluating 100 metaphases (if possible). Chromatid and isochromatid gaps were noted but were not used in calculating the total number of aberrations.

Acceptance criteria: The assay was considered valid if the negative (untreated) and vehicle controls contained < 5% cells with aberrations, the positive control result was significantly higher ( $p \leq 0.01$ ) than that of the vehicle control, a high dose of 10 mM or the highest soluble concentration was used if the material did not cause at least a 50% reduction of the mitotic index at the tested concentrations, and at least 3 concentrations were analyzed.

Data analysis: The statistical analysis employed a Cochran-Armitage test for linear trends and Fisher's Exact Test to compare the percentage of cells with aberrations. Data for polyploidy and/or endoreduplication were also analyzed separately. A test was considered positive if a significant increase in the number of cells with aberrations ( $p \leq 0.01$ ) was observed at one or more concentrations. A dose-response should be observed if there was a significant increase at one or more concentrations.

**Test substance** : Purity of the test material was not confirmed in this study. However, the lot of test material used (062702) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 97.8 %.

**Conclusion Reliability** : Material was not genotoxic under conditions of this assay.  
: (1) valid without restriction  
This was a well-documented OECD guideline study.

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## 5.6 GENETIC TOXICITY 'IN VIVO'

## 5.7 CARCINOGENICITY

### 5.8.1 TOXICITY TO FERTILITY

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

**Species** : Rat  
**Sex** : Male/female

## 5. Toxicity

**Id** 19248-13-6

**Date** 29.10.2003

**Strain** : Sprague-Dawley  
**Route of admin.** : Gavage  
**Exposure period** : 51 days (males), up to 56 days (females)  
**Frequency of treatm.** : Daily  
**Duration of test** : to lactation day 4 -6  
**Doses** : 20, 70 and 200 mg/kg bw/day  
**Control group** : yes, concurrent vehicle  
**NOEL maternal tox.** : = 20 mg/kg bw  
**NOEL teratogen.** : = 200 mg/kg bw  
**NOEL Fetotoxicity** : = 70 mg/kg bw  
**Result** : not developmentally toxic at nonmaternally toxic doses, material was not teratogenic  
**Method** : Other: OECD Guideline 421  
**Year** : 2005  
**GLP** : Yes  
**Test substance** : as prescribed by 1.1 -1.4  
**Remark** : Data and methods pertaining to fertility testing are described in Section 5.8.1.

In a probe study, groups of 3 female rats were treated with 0, 50, 100, 200, or 300 mg/kg bw/day by oral gavage for 11 days and groups of 3 males were dosed with 0, 200, 300 or 400 mg/kg bw/day by oral gavage for 11 days. One male dosed with 300 mg/kg bw and all males dosed with 400 mg/kg bw/day died. One male each from the 300 and 400 mg/kg bw/day groups exhibited clonic convulsions. One female in each of the 200 mg/kg bw/day and 300 mg/kg bw/day groups died. Based on the results, doses of 0, 20, 70 and 200 mg/kg bw/day were chosen for use in the

Investigators reported the results in terms of a NOEL (rather than a NOAEL).

Excursions above the recommended upper limit of 25.0 degrees C for temperature were within acceptable limits (+/- 2 degrees C) and did not constitute a protocol deviation.

The protocol specified use of males weighing 300 +/- 50 g. One male rat in the control group weighed 352.7 g at the beginning of the study. This deviation did not adversely affect the outcome of the study.

**Result** : NOEL: Based on a decrease in weight gain at gestation day 7 in mid and high dose females, the NOEL for parental toxicity is 20 mg/kg. The NOEL for fetotoxicity is 70 mg/kg, based on reductions in litter size, litter weight and body weight at 200 mg/kg. The NOEL for teratogenicity is 200 mg/kg, based on an absence of clinical signs and external malformations in the offspring.

Investigators reported the results in terms of a NOEL (rather than a NOAEL).

Actual dose received: The mean concentrations of test material (determined by GC/FID) in the 20, 70, and 200 mg/ml dosing solutions were 97, 98 and 96% of target concentrations, respectively.

Parental data:

Mortality: Five male (one control, one mid dose and 3 high dose) and five female rats (1 low dose, 3 mid dose and 1 high dose) were either found dead or euthanized for humane reasons.

Clinical observations: Male rats in the high dose group exhibited decreased fecal volume (N= 10 males from days 3-50). All treated males and females exhibited brown, discolored urine throughout the study. Other signs in

treated animals were at similar incidences as controls and were not considered to be related to treatment.

Feed consumption: Mean feed consumption of high dose males was significantly lower ( $p \leq 0.05$ ) than control on Days 7, 42 and 49. Mean feed consumption of high dose females was significantly lower than controls on lactation Day 4.

Body weight: Mean body weights for high dose males were significantly lower ( $p \leq 0.05$ ) than controls on Days 7 through 49, and mean body weight for high dose females was lower than controls on Day 4 of lactation. Mean body weight gains for the high dose male group were significantly lower on Days 7 and 42 than controls. Overall weight gain for the high dose males was 62% of controls. Overall mean weight gain for high dose females during pre-mating and mating was approximately 50% of that of controls and was 12% of that of controls during lactation. Weight gains of mid and high dose females on gestation Days 7 were decreased (62 and 76% of control), but total weight gains during gestation were similar between groups.

Organ weights: Mean absolute testes or epididymis weights were similar between groups. Mean relative testes weights (to body weight) of low dose males were lower and of high dose males were greater than controls.

Pathology: Brown discoloration or staining of the inguinal hair was observed in two high dose males and three high dose females. One control and one high dose male had a focus of mild seminiferous tubule atrophy within the testes. One high dose male had a 4 mm tan mass (sperm granuloma) in the left testis. None of these changes were thought to be related to administration of test material. Three animals that were died or were euthanized early (N=10) exhibited changes in the lungs such as pleuritis (N = 2), and pulmonary edema, vascular thrombosis and bronchitis (N=1), which were thought to be due to gavage error. A fourth rat that did not exhibit any gross lesions was gasping after the first attempt at dosing, and died prior to dosing. The causes of death of the six other animals could not be determined from the pathological examinations.

Reproductive and Developmental Data: There was no effect of treatment on any reproductive parameter measured (reproductive performance, fertility index, fecundity index, pre-coital interval, gestation duration, numbers of implants, numbers of corpora lutea, and post implantation loss). The percentage of pre-implantation loss in high dose animals was higher than control (11.8% vs. 3.8%) but due to the large standard deviation of both groups (15.9% in treated and 11.2% in control), the difference was not significant.

There was no significant effect of treatment on the numbers of live and dead pups on Day 0, live-birth index, or numbers of male and female pups. Litter size for the high dose group was slightly less (but not significantly) than control (14.6 live pups/litter in control vs. 12.2 live pups/litter in treated). This was predominantly due to one litter of seven in one high dose female. The number of live pups from high dose animals was significantly less than control on Day 4 (14.3 per litter in control vs. 9.9 per litter in treated). The percentage of survival from Days 0-4 of high dose pups was decreased (81.4 % vs. 97.3% in control), although not significantly. Mean litter weight and mean pup weight of offspring of high dose animals were significantly lower than control on Days 0 and 4.

Clinical observations observed in pups included bruising/hematomas, pups appearing small, or pups having little or no milk in stomachs. These observations occurred in similar incidences in treated and control animals. There was no significant difference in the incidence of dead or missing

**Test condition**

pups (presumably cannibalized) between treated and control groups.  
: Animals: Male and female Sprague-Dawley rats (CrI:CD9SD)IGS BR) were obtained from Charles River Laboratories, numbered with metal ear tags, and acclimated for 5 days before use. They were randomly allocated by body weight to four groups of 12 animals per sex. The males were 64 days of age and weighed 321.6 +/- 13.3 g (mean +/- SD) and the females were 68 days of age and weighed 228.0 +/- 8.5 g at the start of the study.

Animals were individually housed in suspended wire-mesh cages (except during mating). Solid bottom pans containing bedding material were placed in the cages of the females on Day 19 of gestation. Cages were washed approximately once every 10 days. Animals were housed in an environmentally-controlled room, under a 12 hour light/dark cycle. The actual mean daily temperature and relative humidity ranged from 21.1 – 25.8 degrees C and 37.9 – 65.6%, respectively. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome. No other study was housed in the same room.

Test material: Based on an 11-day probe study, the doses selected for use in the main study were 20, 70, and 200 mg/kg bw/day. Dosing solutions were prepared so that animals from each dose group received equal volumes on a per kg body weight basis. The material was mixed with corn oil to yield concentrations of 20, 70, and 200 mg/ml. Test mixtures were prepared every 3 or 4 days and used within 3 days. Stability of the material in the vehicle was confirmed by analysis of 10 and 200 mg/ml mixtures by gas chromatography/flame ionization detection (GC/FID), 0, 1, 2, 3 and 7 days after mixture preparation. Concentrations of test material in the 10 and 200 mg/ml samples after 7 days were 88% and 85% (respectively) of Day 0 concentrations, indicating that the material was not stable in corn oil for 7 days. The Day 3 concentrations of the 10 and 200 mg/l samples were 104% and 99% (respectively) of Day 0 concentrations, indicating the test material was stable in corn oil for 3 days.

The homogeneity of the mixtures was maintained by using a stirring bar to continuously mix the test solutions while removing aliquots for dosing purposes. Homogeneity of the test material in corn oil was evaluated by measuring the concentration of the test material at three levels (top, middle and bottom) of each dosing preparation. The analytical concentrations in the different layers for the 20 mg/ml mixture varied between 19.45 and 20.94 mg/ml [standard deviation (SD) of 0.37], for the 70 mg/ml mixture varied between 68.19 and 72.03 mg/ml (SD of 1.09), and for the 200 mg/ml mixture varied between 188.8 and 205.9 mg/ml (SD of 5.1). The mixtures were considered homogeneous.

The mean concentrations of test material (determined by GC/FID) in the 20, 70, and 200 mg/ml dosing solutions were 97, 98 and 96% of target concentrations, respectively.

Study conduct: As mentioned previously, the animals were randomly allocated by weight to 4 groups of 12 animals per sex (F0 animals) receiving either 0 (vehicle), 20, 70 or 200 mg/kg bw/day test material. Control animals received a volume of corn oil equal to that administered to test animals. The study consisted of 4 phases: pre mating (14 days), mating (1-14 days), pregnancy (21-23 days) and early lactation (4-6 days). All animals were treated through the day prior to euthanasia, for a total of 51 days for males and 38-56 days for females.

Following 14 days of dosing, males and females were cohabited 1:1 with the same dose group for up to 14 days. Copulation was verified through the identification of sperm in vaginal smears or by the appearance of a copulation plug. The morning copulation was verified was considered Day

0 of gestation. Following copulation, the males and females were housed separately.

Clinical (hands-on) examinations were conducted daily. Animals were observed for moribundity/mortality each weekday afternoon. Clinical observations included (but were not limited to) examination of the hair, skin, eyes, mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic and central nervous systems and behavior.

Feeders were weighed on Days 0, 7 and 14 of the pre-mating phase for all animals. Food consumption was not determined during the mating phase. Feed consumption was measured weekly for males after mating. For females, feeders were weighed on Days 0, 7, 14, and 20 of gestation, and Days 0 and 4 postpartum.

Weights of adult males were measured on Days 0, 7, and weekly thereafter. Adult females were weighed on Days 0, 7, and 14 before mating, Days 0, 7, 14 and 20 of gestation, and Days 0 and 4 postpartum. Body weights of live pups were measured as a group on Days 0 and 4 postpartum.

Adult males were fasted overnight and euthanized with CO<sub>2</sub> on Day 51. Females that delivered a litter and their offspring were euthanized on Days 4 or 5 postpartum. Females showing evidence of copulation but did not deliver on the expected delivery date (Day 21 or 22 of gestation) were euthanized 23, 24 or 25 days after copulation. Females showing no evidence of copulation were euthanized 24-26 days after the last day of the mating period. Terminal weights of adult males (but not females) were measured.

All adult rats were subjected to a necropsy and gross examination. Special attention was paid to the organs of the reproductive system. The uteri from all adult females were examined and implantation sites and corpora lutea were counted. Paired testes and epididymides of males were weighed.

The following tissues were collected from adults and fixed in 10% buffered formalin: ovaries, vagina, uterus, Fallopian tubes, seminal vesicles, coagulating gland, prostate, and gross lesions. The left testis and left epididymis were placed into individual containers, frozen with dry ice, and stored at -70 degrees C. The right testis and right epididymis were fixed in modified Davidson's fluid. After approximately 48 hours, the right testis and right epididymis were transferred into 10% buffered formalin. The ovaries, right testis and right epididymis were examined microscopically for animals in the control and high dose groups. Gross lesions from all dose groups were examined microscopically. Tissue sections (5 or 6 microns) were stained with hematoxylin and eosin before examination. In addition, duplicate sections of testicular tissue were stained with the periodic acid-Schiff procedure and examined.

Statistical evaluations: Statistical evaluations: Adult body weight and body weight change, litter weight, pup body weight and body weight change, feed consumption, organ weights, organ to body weight ratios, pre-coital interval, gestation duration, numbers of implants and corpora lutea, pre and post implantation loss, litter size, live birth index, numbers of live and dead pups, numbers of male and female pups, percent of male pups, and pup survival data were evaluated using Bartlett's test ( $p \leq 0.01$ ), a one-way analysis of variance (ANOVA) ( $p \leq 0.05$ ) and Dunnett's test ( $p \leq 0.05$ ). When the variances of the means were not considered equal by the Bartlett's test, the data were evaluated using a Kruskal-Wallis H Test ( $p \leq 0.05$ ), followed by the Mann-Whitney U Test ( $p \leq 0.05$ ).

**Test substance**

: The purity of the test material was confirmed by gas chromatography with flame ionization detection (GC/FID) to be  $\geq 97.8\%$  prior to use and  $\geq 98.2\%$  at study termination. The structure of the test material was confirmed

## 5. Toxicity

**Id** 19248-13-6  
**Date** 29.10.2003

**Reliability** : using mass spectrometry.  
: (1) valid without restriction  
: OECD Guideline study  
**Flag** : Critical study for SIDS endpoint  
27.07.2005

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### 5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

**Type** : other: combined repeated dose oral toxicity with reproductive/developmental toxicity screening test

**Species** : Rat

**Sex** : male/female

**Strain** : Sprague-Dawley

**Route of admin.** : Gavage

**Exposure period** : 51 days (males), up to 56 days (females)

**Frequency of treatm.** : Daily

**Premating exposure period**

**Male** : 14 days

**Female** : 14 days

**Duration of test** : to lactation day 4 -6

**No. of generation studies** : 1

**Doses** : 20, 70 and 200 mg/kg bw/day

**Control group** : yes, concurrent vehicle

**NOEL parental** : = 20 mg/kg bw

**NOEL F1 offspring** : = 200 mg/kg bw

**Result** : material is not a reproductive toxicant

**Method** : OECD Guideline 421

**Year** : 2005

**GLP** : Yes

**Test substance** : as prescribed by 1.1 -1.4

**Remark** : In a probe study, groups of 3 female rats were treated with 0, 50, 100, 200, or 300 mg/kg bw/day by oral gavage for 11 days and groups of 3 males were dosed with 0, 200, 300 or 400 mg/kg bw/day by oral gavage for 11 days. One male dosed with 300 mg/kg bw and all males dosed with 400 mg/kg bw/day died. One male each from the 300 and 400 mg/kg bw/day groups exhibited clonic convulsions. One female in each of the 200 mg/kg bw/day and 300 mg/kg bw/day groups died. Based on the results, doses of 0, 20, 70 and 200 mg/kg bw/day were chosen for use in the main study.

Investigators reported the results in terms of a NOEL (rather than a NOAEL).

Excursions above the recommended upper limit of 25.0 degrees C for temperature were within acceptable limits (+/- 2 degrees C) and did not constitute a protocol deviation.

The protocol specified use of males weighing 300 +/- 50 g. One male rat in the control group weighed 352.7 g at the beginning of the study. The protocol also specified fixation of the right testis and right epididymis in modified Davidson's fluid for approximately 48 hours. The right testis and right epididymis from one male rat in the 70 mg/kg group were fixed for 69 hours. Both deviations did not adversely affect the outcome of the study.

The slight decrease in epididymal sperm count in 200 mg/kg males had no effect on reproduction and was not associated with any histopathological finding. The presence of brown, discolored urine at 20 and 70 mg/kg did not appear to have any adverse effect on the rats.

**Result** : NOEL: Based on a decrease in weight gain at gestation day 7 in mid and

high dose females, the NOEL for parental toxicity is 20 mg/kg.

Actual dose received: The mean concentrations of test material (determined by GC/FID) in the 20, 70, and 200 mg/ml dosing solutions were 97, 98 and 96% of target concentrations, respectively.

Parental data:

Mortality: Five male (one control, one mid dose and 3 high dose) and five female rats (1 low dose, 3 mid dose and 1 high dose) were either found dead or euthanized for humane reasons.

Clinical observations: Male rats in the high dose group exhibited decreased fecal volume (N= 10 males from days 3-50). All treated males and females exhibited brown, discolored urine throughout the study. Other signs in treated animals were at similar incidences as controls and were not considered to be related to treatment.

Feed consumption: Mean feed consumption of high dose males was significantly lower ( $p \leq 0.05$ ) than control on Days 7, 42 and 49. Mean feed consumption of high dose females was significantly lower than controls on lactation Day 4.

Body weight: Mean body weights for high dose males were significantly lower ( $p \leq 0.05$ ) than controls on Days 7 through 49, and mean body weight for high dose females was lower than controls on Day 4 of lactation. Mean body weight gains for the high dose male group were significantly lower on Days 7 and 42 than controls. Overall weight gain for the high dose males was 62% of controls. Overall mean weight gain for high dose females during pre-mating and mating was approximately 50% of that of controls and was 12% of that of controls during lactation. Weight gains of mid and high dose females on gestation Days 7 were decreased (62 and 76% of control), but total weight gains during gestation were similar between groups.

Organ weights: Mean absolute testes or epididymis weights were similar between groups. Mean relative testes weights (to body weight) of low dose males were lower and of high dose males were greater than controls.

Pathology: Brown discoloration or staining of the inguinal hair was observed in two high dose males and three high dose females. One control and one high dose male had a focus of mild seminiferous tubule atrophy within the testes. One high dose male had a 4 mm tan mass (sperm granuloma) in the left testis. None of these changes were thought to be related to administration of test material. Three animals that were died or were euthanized early (N=10) exhibited changes in the lungs such as pleuritis (N = 2), and pulmonary edema, vascular thrombosis and bronchitis (N=1), which were thought to be due to gavage error. A fourth rat that did not exhibit any gross lesions was gasping after the first attempt at dosing, and died prior to dosing. The causes of death of the six other animals could not be determined from the pathological examinations.

Assessment of Sperm Morphology and Development: No substance-related changes in sperm motility or testicular spermatid counts were observed. Epididymal spermatozoan counts were 15% lower for the 200 mg/kg males compared to controls. This difference was not statistically different, but was considered to be related to treatment.

Reproductive and Developmental Data: There was no effect of treatment on any reproductive parameter measured (reproductive performance, fertility index, fecundity index, pre-coital interval, gestation duration, numbers of implants, numbers of corpora lutea, and post implantation

loss). The percentage of pre-implantation loss in high dose animals was higher than control (11.8% vs. 3.8%) but due to the large standard deviation of both groups (15.9% in treated and 11.2% in control), the difference was not significant.

There was no significant effect of treatment on the numbers of live and dead pups on Day 0, live-birth index, or numbers of male and female pups. Litter size for the high dose group was slightly less (but not significantly) than control (14.6 live pups/litter in control vs. 12.2 live pups/litter in treated). This was predominantly due to one litter of seven in one high dose female. The number of live pups from high dose animals was significantly less than control on Day 4 (14.3 per litter in control vs. 9.9 per litter in treated). The percentage of survival from Days 0-4 of high dose pups was decreased (81.4 % vs. 97.3% in control), although not significantly. Mean litter weight and mean pup weight of offspring of high dose animals were significantly lower than control on Days 0 and 4.

Clinical observations observed in pups included bruising/hematomas, pups appearing small, or pups having little or no milk in stomachs. These observations occurred in similar incidences in treated and control animals. There was no significant difference in the incidence of dead or missing pups (presumably cannibalized) between treated and control groups.

**Test condition**

: Animals: Male and female Sprague-Dawley rats (CrI:CD9SD)IGS BR) were obtained from Charles River Laboratories, numbered with metal ear tags, and acclimated for 5 days before use. They were randomly allocated by body weight to four groups of 12 animals per sex. The males were 64 days of age and weighed 321.6 +/- 13.3 g (mean +/- SD) and the females were 68 days of age and weighed 228.0 +/- 8.5 g at the start of the study.

Animals were individually housed in suspended wire-mesh cages (except during mating). Solid bottom pans containing bedding material were placed in the cages of the females on Day 19 of gestation. Cages were washed approximately once every 10 days. Animals were housed in an environmentally-controlled room, under a 12 hour light/dark cycle. The actual mean daily temperature and relative humidity ranged from 21.1 – 25.8 degrees C and 37.9 – 65.6%, respectively. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome. No other study was housed in the same room.

Test material: Based on an 11-day probe study, the doses selected for use in the main study were 20, 70, and 200 mg/kg bw/day. Dosing solutions were prepared so that animals from each dose group received equal volumes on a per kg body weight basis. The material was mixed with corn oil to yield concentrations of 20, 70, and 200 mg/ml. Test mixtures were prepared every 3 or 4 days and used within 3 days. Stability of the material in the vehicle was confirmed by analysis of 10 and 200 mg/ml mixtures by gas chromatography/flame ionization detection (GC/FID), 0, 1, 2, 3 and 7 days after mixture preparation. Concentrations of test material in the 10 and 200 mg/ml samples after 7 days were 88% and 85% (respectively) of Day 0 concentrations, indicating that the material was not stable in corn oil for 7 days. The Day 3 concentrations of the 10 and 200 mg/l samples were 104% and 99% (respectively) of Day 0 concentrations, indicating the test material was stable in corn oil for 3 days.

The homogeneity of the mixtures was maintained by using a stirring bar to continuously mix the test solutions while removing aliquots for dosing purposes. Homogeneity of the test material in corn oil was evaluated by measuring the concentration of the test material at three levels (top, middle and bottom) of each dosing preparation. The analytical concentrations in the different layers for the 20 mg/ml mixture varied between 19.45 and 20.94 mg/ml [standard deviation (SD) of 0.37], for the 70 mg/ml mixture

varied between 68.19 and 72.03 mg/ml (SD of 1.09), and for the 200 mg/ml mixture varied between 188.8 and 205.9 mg/ml (SD of 5.1). The mixtures were considered homogeneous.

The mean concentrations of test material (determined by GC/FID) in the 20, 70, and 200 mg/ml dosing solutions were 97, 98 and 96% of target concentrations, respectively.

Study conduct: As mentioned previously, the animals were randomly allocated by weight to 4 groups of 12 animals per sex (F0 animals) receiving either 0 (vehicle), 20, 70 or 200 mg/kg bw/day test material. Control animals received a volume of corn oil equal to that administered to test animals. The study consisted of 4 phases: pre mating (14 days), mating (1-14 days), pregnancy (21-23 days) and early lactation (4-6 days). All animals were treated through the day prior to euthanasia, for a total of 51 days for males and 38-56 days for females.

Following 14 days of dosing, males and females were cohabited 1:1 with the same dose group for up to 14 days. Copulation was verified through the identification of sperm in vaginal smears or by the appearance of a copulation plug. The morning copulation was verified was considered Day 0 of gestation. Following copulation, the males and females were housed separately.

Clinical (hands-on) examinations were conducted daily. Animals were observed for moribundity/mortality each weekday afternoon. Clinical observations included (but were not limited to) examination of the hair, skin, eyes, mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic and central nervous systems and behavior.

Feeders were weighed on Days 0, 7 and 14 of the premating phase for all animals. Food consumption was not determined during the mating phase. Feed consumption was measured weekly for males after mating. For females, feeders were weighed on Days 0, 7, 14, and 20 of gestation, and Days 0 and 4 postpartum.

Weights of adult males were measured on Days 0, 7, and weekly thereafter. Adult females were weighed on Days 0, 7, and 14 before mating, Days 0, 7, 14 and 20 of gestation, and Days 0 and 4 postpartum. Body weights of live pups were measured as a group on Days 0 and 4 postpartum.

Adult males were fasted overnight and euthanized with CO<sub>2</sub> on Day 51. Females that delivered a litter and their offspring were euthanized on Days 4 or 5 postpartum. Females showing evidence of copulation but did not deliver on the expected delivery date (Day 21 or 22 of gestation) were euthanized 23, 24 or 25 days after copulation. Females showing no evidence of copulation were euthanized 24-26 days after the last day of the mating period. Terminal weights of adult males (but not females) were measured.

All adult rats were subjected to a necropsy and gross examination. Special attention was paid to the organs of the reproductive system. The uteri from all adult females were examined and implantation sites and corpora lutea were counted. Paired testes and epididymides of males were weighed. The right epididymis was placed in a Petri dish containing 1% bovine serum albumin in phosphate buffered saline warmed to approximately 38 degrees C. The epididymis was pierced with a scalpel blade and sperm were allowed to swim out for at least 3 min. A sample of the mixture in the dish was analyzed with a Hamilton Thorne IVOS automated sperm analyzer. Each motion image from 5 fields was recorded and the percent motility was determined for each animal.

The following tissues were collected from adults and fixed in 10% buffered formalin: ovaries, vagina, uterus, Fallopian tubes, seminal vesicles, coagulating gland, prostate, and gross lesions. The left testis and left epididymis were placed into individual containers, frozen with dry ice, and stored at -70 degrees C. The right testis and right epididymis (after motility analysis) were fixed in modified Davidson's fluid. After approximately 48 hours, the right testis and right epididymis were transferred into 10% buffered formalin. The ovaries, right testis and right epididymis were examined microscopically for animals in the control and high dose groups. Gross lesions from all dose groups were examined microscopically. Tissue sections (5 or 6 microns) were stained with hematoxylin and eosin before examination. In addition, duplicate sections of testicular tissue were stained with the periodic acid-Schiff procedure and examined.

Spermatid head counts were performed on the frozen left testes. After the tissue was thawed, the tunica was removed. The tissue was weighed, homogenized and vortexed. A 100 microliter sample was stained with a dye that stained the head of the sperm. A sample of stained sperm was placed onto a 20 micron slide and analyzed with the automated sperm analyzer. Total sperm counts in 20 fields were determined. The data were reported as total counts (million/organ) and adjusted for organ weight.

Spermatozoa counts were performed on the frozen left epididymis. This tissue was processed in a similar manner as the testicular tissue, except that the caudal left epididymis was used. The data were reported as total counts (million/organ) and adjusted for organ weight.

Statistical evaluations: Adult body weight and body weight change, litter weight, pup body weight and body weight change, feed consumption, organ weights, organ to body weight ratios, precoital interval, gestation duration, numbers of implants and corpora lutea, pre and post implantation loss, litter size, live birth index, numbers of live and dead pups, numbers of male and female pups, percent of male pups, and pup survival data were evaluated using Bartlett's test ( $p \leq 0.01$ ), a one-way analysis of variance (ANOVA) ( $p \leq 0.05$ ) and Dunnett's test ( $p \leq 0.05$ ). When the variances of the means were not considered equal by the Bartlett's test, the data were evaluated using a Kruskal-Wallis H Test ( $p \leq 0.05$ ), followed by the Mann-Whitney U Test ( $p \leq 0.05$ ). Reproductive performance was evaluated using a binary logistic regression ( $p \leq 0.05$ ) followed by a two proportions test ( $p \leq 0.05$ ). Fertility and fecundity indices were evaluated using regression analysis ( $p \leq 0.05$ ), followed by a two proportions test ( $p \leq 0.05$ ).

|                           |   |   |      |
|---------------------------|---|---|------|
| <b>Test substance</b>     | : | The purity of the test material was confirmed by gas chromatography with flame ionization detection (GC/FID) to be $\geq 97.8\%$ prior to use and $\geq 98.2\%$ at study termination. The structure of the test material was confirmed using mass spectrometry. |      |
| <b>Reliability</b>        | : | (1) valid without restriction<br>OECD Guideline study   |      |
| <b>Flag</b><br>27.07.2005 | : | Critical study for SIDS endpoint.   | (14) |

## 5.9 SPECIFIC INVESTIGATIONS

## 5.10 EXPOSURE EXPERIENCE

## 5.11 ADDITIONAL REMARKS

**6.1 ANALYTICAL METHODS**

**6.2 DETECTION AND IDENTIFICATION**

**7.1 FUNCTION**

**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED**

**7.3 ORGANISMS TO BE PROTECTED**

**7.4 USER**

**7.5 RESISTANCE**

**8.1 METHODS HANDLING AND STORING**

**8.2 FIRE GUIDANCE**

**8.3 EMERGENCY MEASURES**

**8.4 POSSIB. OF RENDERING SUBST. HARMLESS**

**8.5 WASTE MANAGEMENT**

**8.6 SIDE-EFFECTS DETECTION**

**8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER**

**8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

- (1) Eastman Chemical Company. 2002. Material Safety Data Sheet for N-(2-Aminoethyl)-N-ethyl-m-toluidine, dated July 15, 2002.
- (2) Eastman Kodak Company. 1964. Laboratory of Industrial Medicine Toxicity Report (unpublished), dated January 3. Notebook Number 63, page 635.
- (3) EPIWIN 3.11 Fugacity Level III Model Program.
- (4) EPIWIN AOP Program (v1.91).
- (5) EPIWIN KOWWIN Program (v1.67).
- (6) EPIWIN MPBPWIN Program (v1.41).
- (7) EPIWIN WSKOW Program (v1.41).
- (8) Hirsch. 2003. N-(2-aminoethyl)-N-ethyl-m-toluidine: A growth inhibition test with the alga, *Selenastrum capricornutum* (unpublished study). Eastman Kodak Company, Health and Environment Laboratories, Study No. EN-512-905403-A, dated June 6, 2003.
- (9) Light GC. 2003. N-(2-aminoethyl)-N-ethyl-m-toluidine: An acute aquatic effects test with the daphnid, *Daphnia magna* (unpublished study). Eastman Kodak Company, Toxicological Sciences Section, Health and Environment Laboratories, Study No. EN-431-905403-A, dated February 26, 2003.
- (10) Light GC. 2003. N-(2-aminoethyl)-N-ethyl-m-toluidine: An acute aquatic effects test with the fathead minnow, *Pimephales promelas* (unpublished study). Eastman Kodak Company, Toxicological Sciences Section, Health and Environment Laboratories, Study No. EN-430-905403-A, dated February 26, 2003.
- (11) Mecchi MS. 2002. Salmonella-Escherichia coli/ mammalian-microsome reverse mutation assay with a confirmatory assay with EC2002-0209 (unpublished study). Covance Laboratories Inc. Study Number 24263-0-409OECD, dated December 23, 2002.
- (12) Moulton ME. 2003. N-(2-aminoethyl)-N-ethyl-m-toluidine: Determination of ready biodegradability (biotic degradation) using the CO2 evolution test (modified Sturm)(unpublished study). Eastman Kodak Company, Toxicological Sciences Section, Health and Environment Laboratories, Study No. EN-113-904403-A, dated February 25, 2003.
- (13) Murli H. 2003. Chromosomal aberrations in Chinese Hamster Ovary (CHO) Cells with EC2002-0209 (unpublished study). Covance Laboratories Inc. Study Number 24263-0-437OECD, dated January 27.
- (14) Shepard KP, Murphy JF (2005). N-(2-aminoethyl)-N-ethyl-m-toluidine, Synonyms: N-ethyl-N-(3-methylphenyl)-1,2-ethanediamine; Amine 3. Reproduction /developmental toxicity screening test in the rat. Toxicological Sciences Laboratory, Health and Environment Laboratories Project ID 2002209G2, dated July 15, 2005 (unpublished study).