

201-15494B

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PROTECTED
CONFIDENTIAL

I U C L I D

Data Set

Existing Chemical : ID: 63133-74-4
CAS No. : 63133-74-4
Substance name : Ethyl(3-methylphenyl)-amino acetonitrile
Molecular Formula : C₁₁H₁₄N₂

Producer related part
Company : Eastman Chemical Company
Creation date : 17.09.2003

Substance related part
Company : Eastman Chemical Company
Creation date : 17.09.2003

Status :
Memo :

Printing date : 08.12.2003
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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 63133-74-4
Date 14.07.2004

1.0.1 APPLICANT AND COMPANY INFORMATION

Type : manufacturer
Name : Eastman Chemical Company
Contact person :
Date :
Street :
Town : Kingsport Tennessee
Country : United States
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

Reliability : (1) valid without restriction
23.10.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name :
Smiles Code : N#CCN(c(ccc1C)c1)CC
Molecular formula : C11H14N2
Molecular weight : 174.27
Petrol class :

23.10.2003 (2)

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : other: typical for manufactured product
Substance type : organic
Physical status : liquid
Purity : ca. 98 % w/w
Colour : colorless
Odour : odorless

23.10.2003 (2)

1.1.2 SPECTRA

1. General Information

Id 63133-74-4
Date 14.07.2004

1.2 SYNONYMS AND TRADENAMES

N-Cyanomethyl-N-ethyl-m-toluidine

N-ethyl-m-toluidinoacetonitrile

N-ethyl-N-cyanomethyl-m-toluidine

Nitrile 3

Nitrile III

1.3 IMPURITIES

Purity : typical for substance
CAS-No : 102-27-2
EC-No : 203-019-4
EINECS-Name : N-ethyl-m-toluidine
Molecular formula : C₉H₁₃ N
Value : ca. 2 % w/w

Reliability : (1) valid without restriction
23.10.2003 (2)

1.4 ADDITIVES

Remark : No additives
23.10.2003

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. Physico-Chemical Data

Id 63133-74-4
Date 29.10.2003

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.

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 about 98%.

Flag : Critical study for SIDS endpoint
08.12.2003

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.

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 about 98%.

Flag : Critical study for SIDS endpoint
23.10.2003

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : ca. 0.027 hPa at 25 °C
Method : other (calculated) by EPIWIN MPBPwin
Year : 2003
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Remarks : The inputs to the model were CAS No. 63133-74-4, a melting point of 0 and a boiling point of 250 degrees C.

Reliability : (2) valid with restrictions

2. Physico-Chemical Data

Id 63133-74-4
Date 29.10.2003

Flag : Data were obtained by modeling.
23.10.2003 : Critical study for SIDS endpoint (7)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 2.73 at 25 °C
pH value : = 7
Method : other (calculated)
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : The inputs to the model were CAS No. 63133-74-4, a melting point of 0 and a boiling point of 250 degrees C.

Reliability : (2) valid with restrictions
Data were obtained using a model estimation program.

Flag : Critical study for SIDS endpoint
23.10.2003 (6)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : water
Value : = 252 mg/l at 25 °C
pH value : = 7
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

Remark : The inputs to the model were CAS No. 63133-74-4, a melting point of 0 and a boiling point of 250 degrees C.

Reliability : (2) valid with restrictions
Data were obtained by modeling.

Flag : Critical study for SIDS endpoint
23.10.2003 (8)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2. Physico-Chemical Data

Id 63133-74-4
Date 29.10.2003

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 : = 0.000000002106399 cm³/(molecule*sec)
 Degradation : = 50% after 0.6 hour(s)
 Deg. product :
 Method : other (calculated)
 Year : 2003
 GLP : No
 Test substance : as prescribed by 1.1 - 1.4

Remark : The inputs to the model were CAS No. 63133-74-4, 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

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3.1.2 STABILITY IN WATER

Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN Hydrowin is not set up to estimate a hydrolysis rate constant for organic nitriles, such as the current test substance.

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Test substance : as prescribed by 1.1 - 1.4

Remark : The structural features of nitrile 3 for consideration of susceptibility to hydrolysis include an aromatic methyl group, an aromatic dialkyl-substituted tertiary amine, and a primary alkyl nitrile. Aromatic methyl groups and tertiary aromatic amines 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. CH₃ and NR₂ are extremely poor leaving groups, and the corresponding acid salt of a tertiary amine is 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).

Alkyl nitriles may be hydrolyzed to give amides or carboxylic acids under the conditions of either acid or base catalysis (3,4). However, the requisite conditions are severe to effect hydrolysis. Acid hydrolysis typically requires 75% aqueous sulfuric acid at 160°C, and alkaline hydrolysis requires 30-40% aqueous sodium hydroxide at reflux. The requisite severity of the conditions for cyano group hydrolysis establishes the resistance of the cyano moiety of nitrile 3 to simple hydrolysis under conditions of moderate temperature and pH.

Based upon the physical and chemical properties of nitrile 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 nitrile 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.

(3) March, J., ed. "Advanced Organic Chemistry", 3rd edition, pp. 788-789, John Wiley & Sons, New York, 1985.

(4) Rappoport, Z., ed. "The Chemistry of the Cyano Group", 1st edition, pp. 168-169, 256-262, Interscience Publishers, London, 1970.

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 : 0.0836 % (Fugacity Model Level III)
Water : 31.4 % (Fugacity Model Level III)
Biota : 0.333 % (Fugacity Model Level II/III)
Soil : 68.2 % (Fugacity Model Level II/III)
Method : other: calculated
Year : 2003

Remark : The inputs to the model were CAS No. 63133-74-4, 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 : EPIWIN Henry Program (v3.10) calculates a Henry's Law Constant of 5.21E-008 atm-m³/mol using the Bond Estimate method.

Reliability : (2) valid with restrictions
The data were obtained using a model estimation program.

Flag : Critical study for SIDS endpoint

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3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other: activated sludge
Concentration	:	20 mg/l related to DOC (Dissolved Organic Carbon) related to
Contact time	:	
Degradation	:	= 9 - 13 (±) % after 28 day(s)
Result	:	other: not readily biodegradable
Kinetic of testsubst.	:	1 day(s) 0 - 1 % 10 day(s) 0 - 3 % 14 day(s) 4 - 6 % 20 day(s) 8 - 12 % 28 day(s) 9 - 13 %
Control substance	:	Benzoic acid, sodium salt
Kinetic	:	14 59 % 28 65 %
Deg. product	:	
Method	:	OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO ₂ evolution)"
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	According to a GLP study, the solubility of the test material in dilution water used for aquatic tests (at 20 degrees C) is 136 mg/l at 2 hours and 246 mg/l at 24 hours. It is assumed that the solubility limit of the test material in the medium used in this biodegradation study is similar to these values. 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.
Result	:	Biodegradation of the test material began in one of the vessels (vessel 1) at day 0 and in the other between days 10 and 14. Biodegradation in vessel 1 reached 10% on day 17. By day 28, the amount of material in vessels 1 and 2 had degraded by 13% and 9%, respectively. The material was not readily biodegraded under the test conditions. The positive control was 59% degraded at day 10 and 65 % degraded at day 28. The pH of the BSM at the beginning of the test was 7.489. The pH ranged from 7.450 - 7.884 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 ₂ (25.4 mg CO ₂ /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 ₂ after scrubbing. The average temperature was 22 +/- 0.5 degrees C.
Test condition	:	Test bacteria: Activated sludge microorganisms were obtained from a domestic wastewater treatment plant. Upon arrival at the test site, the

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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 E 6 organisms/ml) were estimated using a dip slide.

Test solutions: Stock solutions of phosphate buffer (8.50 g KH₂PO₄, 21.75 g K₂HPO₄, 33.4 g Na₂HPO₄·2H₂O, and 0.50 g NH₄Cl in 1000 ml distilled water), calcium chloride solution (27.50 g CaCl₂ in 1000 ml distilled water) and magnesium sulfate solution (22.50 g MgSO₄·7H₂O in 1000 ml distilled water) were made in advance, filter sterilized and refrigerated until needed (up to 6 months). A solution containing 25 mg FeCl₃·6H₂O 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 C.

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 CO₂ scrubbing apparatus was set up to remove CO₂ (at a constant rate) from the air supplied to the test vessels. The air was diverted through a drying column (containing Drierite), a CO₂ 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)₂] 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 CO₂.

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 CO₂-free air for approximately 24 hours to purge the system of CO₂. After the aeration period, test material (26.4 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 CO₂ 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)₂.

At the beginning of the test, CO₂-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 CO₂ 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)₂ 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 CO₂ produced. The percentage of material biodegraded was calculated as the mg CO₂ produced x 100 / theoretical CO₂ x mg of test material used. The theoretical CO₂ = number of carbon atoms in the test material x MW of CO₂ / MW of the test material. The CO₂ 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 > = 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 CO₂ evolution in the blank was not > 40 mg CO₂/l, and 5) the CO₂ was scrubbed from the incoming airline.

Test substance : Purity of the material was determined to be 99.3 % (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

23.09.2003 (13)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	: semistatic
Species	: Pimephales promelas (Fish, fresh water)
Exposure period	: 96 hour(s)
Unit	: mg/l
LC0	: = 21.5 measured/nominal
LC50	: = 27.7 measured/nominal
LC100	: = 35.6 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

Remark : According to a GLP study, the solubility of the test material in dilution water used for aquatic tests (at 20 degrees C) is 136 mg/l at 2 hours and 246 mg/l at 24 hours. It is assumed that the solubility limit of the test material in the water used in this study is similar to these values.

Result : None of the control fish or fish exposed to 9.1 or 14 mg/l died or had abnormal behavior during the study. All of the fish exposed to 21.5 mg/l survived, but had depressed activity after 4 hours of exposure (which persisted to the end of the study). All fish exposed to 35.6 or 58.8 mg/l died after 4 hours of exposure. The 24, 48, 72 and 96 hour LC50 values were 27.7 mg/l. The highest concentration causing no (< = 10%) mortality was 21.5 mg/l. The lowest concentration causing 100% mortality was 35.6 mg/l.

Throughout the study, the temperatures of the solutions were maintained at 20 degrees C and the pH values ranged from 8.1 to 8.3. Dissolved oxygen concentrations of the control and test solutions ranged from 8.3 - 8.8 mg/l and 8.3 - 8.9 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 10, 15, 22.5, 33.8 and 50.6 mg/l. Corresponding analytical concentrations were 9.1, 14, 21.5, 35.6 and 58.8 mg/l, respectively. The amount of test material lost from the solutions containing 9.1, 14 or 21.5 mg/l ranged from 6.4% to 16.3% over 24 hours. For the first and second 24 hour periods, the amount of material lost did not appear to be dependent on concentration. For the third and fourth 24 hour periods, more material was lost from the solution containing 21.5 mg/l (16.0 - 16.3%) than the solutions containing 14 mg/l (12.8 - 12.9%) or 9.1 mg/l (6.4 - 12.1%). Loss of material over 24 hours was not determined for the 35.6 and 58.8 mg/l concentrations, since all fish exposed to these concentrations died by 4 hours.

Test condition : 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 (0.10 and 0.15 g/set) and mean standard length (1.9 and 2.1 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 CaCO₃) 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), 10, 15, 22.5, 33.8 and 50.6 mg/l were prepared by directly adding the appropriate amounts of test material (0, 0.2, 0.3, 0.45, 0.68 and 1.01 g) to separate test vessels (Pyrex cuboidal glass tanks) containing 20 liters of dilution water. The approximate headspace was 2700 cm³. The solutions were stirred with a hand-held mixer for 2-3 min, then mixed for an additional hour with a stir bar on a stir plate. The solutions settled for 15 minutes before measurement of physical parameters.

Test conduct: The test was performed as a 96-hour semi-static exposure. Fish were added directly to the test solutions, which were prepared in duplicate. Test solutions were renewed after each 24 hour period, maintained at 20 +/- 1 degrees C, and illuminated as previously described. The fish were placed into 1 liter beakers containing seasoned solutions until fresh solutions were prepared. 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 start 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 fresh and seasoned exposure solutions were collected at the start and end of each 24 hour interval and analyzed for concentration of test material using gas chromatography with flame ionization detection. Samples analyzed contained equal volumes of solution from corresponding replicates.

Analysis of data: The concentrations of material in the solutions were determined by calculating the geometric mean of the corresponding fresh and seasoned solutions for each 24 hour interval and averaging the corresponding 24 hour values. Statistical analyses were performed using TOXSTAT statistical software. The LC50 values at 24, 48, 72 and 96 hours were calculated using the Trimmed Spearman-Kärber method.

The test was considered valid if control mortality was <= 10%, dissolved oxygen did not fall below 60% of the initial oxygen level, the temperature was 20 +/- 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 99.3 % (weight percent) by gas chromatography with flame ionization detection. The

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Reliability : structure was 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 could affect the outcome of the test.

Flag : Critical study for SIDS endpoint

23.09.2003

(11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : semistatic
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 21.7 measured/nominal
EC50 : = 40 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

Remark : According to a GLP study, the solubility of the test material in dilution water used for aquatic tests (at 20 degrees C) is 136 mg/l at 2 hours and 246 mg/l at 24 hours. It is assumed that the solubility limit of the test material in the water used in this study is similar to these values.

Result : None of the control daphnids or daphnids exposed to 9.1 or 14 mg/l were immobile or had abnormal behavior during the study. None of the daphnids exposed to 21.7 mg/l were immobile after 24 hours. However, 10% immobility was observed in this group at 48 hours. After 24 and 48 hours, immobility was noted in 25% and 30% of daphnids exposed to 34.1 mg/l, and 55% and 75% of daphnids exposed to 51.6 mg/l. The 24 and 48 hour EC50 values were 47.6 and 40.0 mg/l, respectively. The highest concentration causing no (< = 10%) immobility was 21.7 mg/l. The lowest concentration causing 100% could not be determined since none of the tested concentrations killed all of the organisms. Results for the two replicates per concentration were consistent.

Throughout the study, the temperatures of the solutions were maintained at 20 degrees C and the pH values ranged from 8.2 to 8.3. Dissolved oxygen concentrations of the control and test solutions ranged from 8.5 - 8.8 mg/l and 8.4 - 8.8 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 10, 15, 22.5, 33.8 and 50.6 mg/l. Corresponding analytical concentrations were 9.1, 14, 21.7, 34.1 and 51.6 mg/l, respectively. The amount of test material lost from the solutions ranged from 5% to 21% over 24 hours. The amount of material lost did not appear to be dependent on concentration.

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 CaCO₃) 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), 10, 15, 22.5, 33.8 and 50.6 mg/l were prepared by directly adding the appropriate amounts of test material (0, 0.2, 0.3, 0.45, 0.68 and 1.01 g) to separate vessels (Pyrex cuboidal glass tanks) containing 20 liters of dilution water. The approximate headspace was 2700 cm³. The solutions were stirred with a hand-held mixer for 2-3 min, then mixed for an additional hour with a stir bar on a stir plate. The solutions settled for 15 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 cm³.

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 semi-static exposure. Test solutions were renewed after each 24 hour period, maintained at 20 +/- 1 degrees C, and illuminated as previously described. Daphnia were transferred by pipette from the seasoned solutions to the fresh solutions. 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 fresh and seasoned exposure solutions were measured at the start and end of each 24-hour period. The appearances of the exposure solutions at 0, 4, 24 and 48 hours were noted. Samples of the fresh and seasoned exposure solutions were collected at the start and end of each 24 hour interval and analyzed for concentration of test material using gas chromatography with flame ionization detection. Samples analyzed contained equal volumes of solution from corresponding replicates.

Analysis of data: The concentrations of material in the solutions were determined by calculating the geometric mean of the corresponding fresh and seasoned solutions for each 24 hour interval and averaging the corresponding 24 hour values. Statistical analyses were performed using TOXSTAT statistical software. The EC50 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

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- Test substance** : influence the outcome.
: Purity of the material was determined to be 99.3 % (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 could 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 : = .68
EbC50 : = 1.40
ErC50 : = 2.88
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

- Remark** : The solubility of the test material in algal medium was determined to be 88.3 mg/l at 2 hours and 169 mg/l at 24 hours using a timed stir test method.

- Result** : The protocol stipulated that the test material concentration was to remain within 80% of the initial concentration throughout the study. During the study, loss of test material ranged from 23.1 – 59.5%. Losses from flasks containing no algae were similar to those containing algae, suggesting that the material evaporated. Study personnel concluded that this deviation did not impact the outcome of the test.
- : Exposure to 0.37 and 0.68 mg/l had no significant effect on biomass or growth rate at any time point. Exposure to > = 1.37 mg/l caused a dose and time - dependent inhibition of biomass and growth rate. Exposure to 1.37 mg/l caused a 20% reduction in growth rate and a 44% reduction in biomass by 72 hours. The growth rate and biomass of cells incubated with 3.16 mg/l were reduced by 59% and 87 % by 72 hours, respectively. The growth rate and biomass of cells exposed to 6.51 mg/l were reduced by 92% and 87% by 72 hours, respectively. The EbC50 (0-72 hour) value was 1.40 mg/l, and the ErC50 (0-72 hour) value was 2.88 mg/l. The 72 hour NOEC value was 0.68 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 0.37, 0.68, 1.37, 3.16 and 6.51 mg/l. The amount of material lost over the course of the experiment in flasks containing algae ranged from 23.1 – 59.5%. The amount of material lost was concentration-dependent. The light-exposed and light-shielded controls lost 63.3% and 51.9% test material over 72 hours, respectively. These results indicated that the test material was not stable in the medium.

The mean temperature and illumination were 24 degrees C and 742 foot-candles (range 738 - 746 foot-candles) throughout the test. The pH of test

Test condition

and control solutions ranged from 7.51 – 7.88. 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 123-fold within 72 hours. The control cells exhibited normal log growth.

: Test Organisms: A 4-day culture of *Selenastrum capricornutum* (passage 4 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 (0.0499 g) was added to 498.96 g of algal growth medium (to produce a nominal concentration of 100 mg/l). The solution was stirred and sonicated to fully dissolve the test material. The solution was vacuum filtered through a sterile 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 (1.0 ml) 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. One hundred milliliters of the highest concentration (10 mg/l) were added to 5 flasks and 100 ml of the remaining test concentrations were added to 3 flasks each. Test medium (100 ml) that did not contain test material (medium control) was added to 3 flasks. Algae (337 microliters of algal stock culture to achieve an initial cell density of 1×10^4 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 742 +/- 3.4 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 inhibition 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

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Test substance : cultures increased by a factor of at least 16 within 72 hours.
: Purity of the material was determined to be 99.3 % (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

Type : LD50
Value : = 200 - 400 mg/kg bw
Species : rat
Strain : other: not listed
Sex : no data
Number of animals : 8
Vehicle : other: corn oil
Doses : 100 - 800 mg/kg bw
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.

Result : Weight change over two weeks is listed as 4+. It is not known if this is the average number of grams gained, or a value chosen from a scale.
 : The LD50 value was 200 - 400 mg/kg. The number of animals that died in each group was not listed. The time of death was 3 hours - 1 day after treatment. Autopsy results were negative.

Test condition : Symptoms of toxicity were weakness, vasodilation, sides caved in, and ataxia. Rats given 200 mg/kg did not form methemoglobin. Animals gained an average of 4 g over 2 weeks.
 : A 10% solution of the test material in corn oil was given by gavage to 8 rats at concentrations ranging from 100 -200 mg/kg. Mortality and symptoms of toxicity were monitored over a 14 day period. Weight change over 14 days was calculated.

Test substance : Purity of test material was not mentioned.

Reliability : (2) valid with restrictions
 Basic data are given. The method of calculating the LD50 value was not listed. The number of animals in each treatment group was not mentioned. The sex of the animals is unknown.

(3)

Type : LD50
Value : = 400 - 800 mg/kg bw
Species : mouse
Strain : other: not listed
Sex : no data
Number of animals : 10
Vehicle :
Doses : 50 - 800 mg/kg bw
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 50, 100, 200, 400 and 800 mg/kg/ test material.

Result : The LD50 value was 50 - 800 mg/kg. The number of animals that died in each group was not listed. The time of death was 1 day after treatment.

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Symptoms of toxicity were weakness, vasodilation, rolling, convulsions when handled, tremor and rough coat. Animals gained an average of 8 g over 2 weeks.

Test condition : Undiluted test material was given by gavage to 10 mice at concentrations ranging from 50 -800 mg/kg. Mortality and symptoms of toxicity were monitored over a 14 day period. Weight change over 14 days was calculated.

Test substance : Purity of test material was not mentioned.

Reliability : (2) valid with restrictions
Basic data are given. The method of calculating the LD50 value was not listed. The number of animals in each treatment group was not mentioned. The sex of the animals is unknown.

(3)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 20 ml/kg bw
Species : guinea pig
Strain : other: not listed
Sex : no data
Number of animals : 3
Vehicle :
Doses : 5 - 20 ml/kg
Method : other
Year : 1964
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : None of the animals died. The only symptom of toxicity noted was slight edema and erythema, which abated by 1 week. It is not known if this was caused by all three concentrations, or only the highest one used.

Animals gained an average of 66.7 g over 14 days.

Test condition : Undiluted test material was administered dermally (under a cuff) to 3 guinea pigs at concentrations ranging from 5 – 20 ml/kg. Mortality and symptoms of toxicity were monitored over a 14 day period. Weight change over 14 days was calculated.

Test substance : Purity of test material was not mentioned.

Reliability : (2) valid with restrictions
Basic data are given. The number of animals in each treatment group was not mentioned, but is assumed to be one. The sex of the animals is unknown.

(3)

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
System of testing	: Salmonella typhimurium/TA98, 100, 1535, 1537, and Escherichia coli/WP2uvrA(pKM101)
Test concentration	: 1.00, 3.33, 10.0, 33.3, 100, 333 and 1000 micrograms/plate (Salmonella without S-9), 3.33, 10.0, 33.3, 100, 333, 1000 and 5000 micrograms/plate (Salmonella with S9), and 10.0, 33.3, 100, 1000, 3330 and 5000 micrograms/plate (E. coli)
Cytotoxic concentr.	: 333 micrograms/plate (Salmonella with or without S9), 1000 micrograms/plate (E. coli with S9) and 3330 micrograms/plate (E. coli without S9)
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 : A preliminary solubility test showed that the material was not soluble in water but was soluble in DMSO at a concentration of 100 mg/ml.

In the first mutagenicity test, no positive increases were observed in the mean number of revertants per plate with any of the strains incubated with test material in either the presence or absence of S9 mix. Cytotoxicity was observed in the Salmonella strains at concentrations of 100 to 333 micrograms/plate in the presence or absence of S9 mix. Cytotoxicity was observed in E. coli strain WP2uvrA at ≥ 1000 micrograms/plate in the presence or absence of S9 mix. No precipitate was observed in any of the plates.

In the confirmatory mutagenicity test, a 3.0-fold increase in revertants was observed in Salmonella strain TA1537 incubated with test material (at 3.33 and 33.3 micrograms/plate) in the absence (but not presence) of S9. This increase was not concentration-dependent, and the number of revertants observed (6) was within the range of historical controls (1-18). An increased number of revertants was not noted in any of the other strains incubated with any concentration of test material (either in the absence or presence of S9). Cytotoxicity was noted in Salmonella strains at concentrations ≥ 333 micrograms/plate (with or without S9). Cytotoxicity also was observed in E. coli strain WP2uvrA incubated with ≥ 1000 micrograms/plate with S9 or 3330 micrograms/plate without S9. No precipitate was observed in any of the plates.

In the third experiment performed only with Salmonella strain TA1537 in the absence of S9, there was no increase in the mean number of revertants per plate in cells exposed to test material.

The positive result in strain TA1537 in the second study was not considered to be related to test material since it was not reproduced.

Test condition

Therefore, the results of the studies were considered to be negative. All three tests were valid, since they met all criteria for a valid study.

: 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 x 10E9 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 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 the first test (1.00, 3.33, 10.0, 33.3, 100, 333 and 1000 micrograms/plate for *Salmonella* without S-9; 3.33, 10.0, 33.3, 100, 333, 1000 and 5000 micrograms/plate for *Salmonella* with S9; and 10.0, 33.3, 100, 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). The maximum concentration tested in the first study was eliminated for the confirmatory test based on cytotoxicity. All concentrations used in the first test were retested in a third study performed only with *S. typhimurium* strain TA1537 in the absence of S9 (with the exception of 1.00 micrograms/plate).

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 and 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 +/- 2 degrees C. This was overlaid onto 25 ml of

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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 +/- 4 hours of inverted incubation at 37 +/- 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, and a third experiment was conducted in strain TA1537 in the absence of S9.

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 constitute a valid assay (test strain integrity, characteristic number of spontaneous revertants, cell density $\geq 0.5 \times 10^9$, 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 (062602) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 99.3 %.

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

Reliability : (1) valid without restriction
This was a well-documented guideline study conducted under GLP assurances.

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(12)

Type : Chromosomal aberration test

System of testing : Chinese Hamster Ovary (CHO) Cells

Test concentration : up to 600 micrograms/ml

Cytotoxic concentr. : 300 - 400 micrograms/ml

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, a precipitate was noted in cells treated with 600 micrograms/ml. A slight precipitate was noted in cells treated with 420 micrograms/ml. Reductions of 9%, 9%, 8%, 0%, 29%, 69% and 97% were observed in the mitotic indices of the cultures treated with 70.7, 101, 144, 206, 294, 420 and 600 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 144, 206, 294, and 420 micrograms/ml.

In the confirmatory study without activation, a precipitate was noted in cells treated with 100, 150, 225, 300 and 400 micrograms/ml. Debris and a reduced number of dividing cells were found in cells treated with concentrations ≥ 150 micrograms/ml. Reductions of 16%, 11%, 10%, 10%, 51%, 74%, 60%, 91% and 100% were observed in the mitotic indices of cultures treated with 6.25, 12.5, 25.0, 50.0, 100, 150, 225, 300 and 400 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, a precipitate was noted in cultures treated with 600 micrograms/ml. Only dead cells were observed at this concentration. A slight precipitate was found in cells treated with 420 micrograms/ml. Reductions of 9%, 11%, 26%, 60% and

85% were observed in the mitotic indices of cultures treated with 101, 144, 206, 294 and 420 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 101, 144, 206 and 294 micrograms/ml. No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in analyzed cultures (except for a weakly significant increase in endoreduplication at the 294 micrograms/ml). This was believed to be a statistical anomaly due to the vehicle controls having 0% endoreduplication. The result (4.5%) was within the historical negative and vehicle control data for endoreduplication (0-5.5% and 0-6%, respectively).

In the confirmatory study with metabolic activation, a precipitate was observed in medium of cells incubated with 100, 150, 225, 300 or 400 micrograms/ml. Unhealthy and/or dead cells were observed in cultures treated with 300 or 400 micrograms/ml. Reductions of 0%, 0%, 0%, 0%, 0%, 41% and 86% were observed in the mitotic indices of cultures treated with 25, 50, 100, 150, 225, 300 and 400 micrograms/ml, respectively. Chromosomal aberrations were analyzed from cultures treated with 100, 150, 225 and 300 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 confirmatory test with 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.

Test condition

: 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 46.8, 93.5, 187 and 374 mg/ml appeared transparent and colorless. These solutions (10 microliters/ml) were tested for solubility in culture medium. Concentrations of 468 to 3740 micrograms/ml precipitated in the medium. Therefore, the highest concentration to be used in the tests was 600 micrograms/ml. 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 7.50 and 12.5 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 (4.08, 5.83, 8.33, 11.9, 17.0, 24.3, 34.7, 49.5, 70.7, 101, 144, 206, 294, 420 and 600 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 16.8 hours. Colcemid (0.1 micrograms/ml) was present during the last 2 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 (25.0, 50.0, 100, 150, 225, 300 and 400 micrograms/ml). In the test without metabolic activation, the test material (3.13, 6.25, 12.5, 25.0, 50.0, 100, 150, 225, 300 and 400 micrograms/ml), positive control and negative controls were incubated with the cells for 19.8 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 ($p < 0.01$) 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 ($p < 0.01$) was observed at one or more concentrations. A dose-response also 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 (062602) was the same as that used in the aquatic toxicity studies, where the purity was analyzed to be 99.3 %.

Conclusion Reliability

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

23.09.2003

(14)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

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	:	25, 75 and 150 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 25 mg/kg bw
NOAEL F1 offspring	:	= 150 mg/kg bw
Method	:	OECD Guideline 421
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 -1.4
Remark	:	In a probe study, groups of female rats were treated with 25, 50, 100, 200, 300 or 400 mg/kg bw/day by oral gavage for up to 11 days. One 400 mg/kg bw/day rat was euthanized in extremis after 6 doses. Systemic toxicity was noted at doses \geq 200 mg/kg bw/day. 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 (\pm 2 degrees C) and did not constitute a protocol deviation.
Result	:	NOEL and LOEL: The NOEL for systemic toxicity in males was 25 mg/kg bw/day, based on reductions in body weight at 75 mg/kg bw/day. The NOEL for systemic and reproductive toxicity in females and reproductive toxicity in males was 150 mg/kg bw/day. Actual dose received: The mean concentrations of test material (determined by GC/FID) in the 25, 75, 150 and 250 mg/ml dosing solutions were 99.2, 103, 102 and 106% of target concentrations, respectively. Parental data: Mortality: One control male as found dead on Day 19. One 75 mg/kg bw/day male and one 150 mg/kg bw/day female were euthanized on Days 44 and 20 (respectively) for humane reasons. Clinical observations:

150 mg/kg bw/day: Reduced activity, vasodilation, rapid respiration, and fur wet with saliva along with transient convulsions (chewing, clonic and/or tonic) and tremors were observed in most males. Most females also showed fur wet with saliva and vasodilation, and a single animal had a single incidence of reduced activity.

75 mg/kg bw/day: Transient vasodilation was observed in all males and one female, which abated the following morning. One male and one female also had a single observation of fur wet with saliva. Reduced activity, unkempt hair coat, urine-stained fur, shallow respiration, decreased fecal volume, porphyrin discharges from the eye and red discoloration of the peri-oral fur were observed in the male that was euthanized.

25 mg/kg bw/day: No clinical observations were noted.

Feed consumption: No differences in feed consumption were noted in treated males. Feed consumption was higher ($p \leq 0.05$) on Day 14 of the pre-mating phase and on Day 7 of gestation in 75 mg/kg bw/day females than controls.

Body weight:

150 mg/kg bw/day: Mean body weights for males were lower ($p \leq 0.05$) than control from Day 7 to study termination. Mean body weight gains were lower ($p \leq 0.05$) for the males on Days 7 and 25. There was no effect of treatment on body weights or body weight gains in females treated with this or lower doses.

75 mg/kg bw/day: Mean body weights for males were lower ($p \leq 0.05$) than control from Day 7 to study termination. Mean body weight gains were lower ($p \leq 0.05$) for the males on Days 7.

Organ weights: Mean absolute testes weights were lower ($p \leq 0.05$) for the 150 mg/kg bw/day group than controls. Relative testes to body weight ratios were similar in treated animals than controls.

Pathology: All of the gross observations were noted in 2 male and 2 female rats. None of them were related to treatment. They were considered to either represent spontaneous disease processes or gavage error. Two 150 mg/kg bw/day males had minimal seminiferous tubule atrophy (one tubule in each testis), which was within expected background and not considered to be substance-related. All other organs were normal in appearance.

Assessment of Sperm Morphology and Development: No substance-related changes in sperm motility, epididymal spermatozoa counts or testicular spermatid counts were observed.

Reproductive and Developmental Data: There was no effect of treatment on any reproductive or developmental parameter measured (reproductive performance, fertility index, fecundity index, precoital interval, gestation duration, numbers of implants, numbers of corpora lutea, pre and post implantation loss, pup survival, live and dead pups, male and female pups and pup body weight). Clinical observations observed in pups included bruising/hematomas, pups appearing small, pups having little or no milk in stomachs, darker in color, and pale. These observations occurred in similar incidences in treated and control animals. There was no 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 +/- 10.3 g (mean +/- SD) and the females were 67 days of age and weighed 233.6 +/- 9.7 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 36.3 – 54.9%, respectively. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome. Feeders were changed and sanitized once per week.

Test material: Based on an 11-day probe study in female rats, the doses selected for use in the main study were 25, 75, and 250 mg/kg bw/day. The original high dose of 250 mg/kg bw/day was reduced to 150 mg/kg bw/day in the main study after the first day of dosing because of excessive mortality in male rats (4/12 within 4 hours of dosing). For the low and mid dose groups, the test material mixtures were prepared once based on the stability of the mixture. Changing the dose level for the high dose group to 150 mg/kg bw/day resulted in the mixtures being prepared three times during the study (pre-study, Day 0 and Day 1).

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 25, 75, 150 and 250 mg/ml. Stability of the test material in corn oil was determined by repeated analysis of 25, 75 and 250 mg/ml mixtures of the test substance. Mixtures of 25 mg/ml were analyzed using gas chromatography/flame ionization detection (GC/FID) on 0, 1, 2, 3, 55 and 69 days. Mixtures of 75 and 250 mg/ml were analyzed 0, 1, 2, 3, 4, 7, 14, 28, 51 and 70 days after test mixture preparation. Concentrations of test material were 26.9 +/- 1.53, 76.6 +/- 1.38 and 257 +/- 5.0 mg/ml (mean +/- SD) prior to storage and were 25.8 +/- 0.96, 81.1 +/- 4.02 and 253 +/- 8.5 mg/ml after 69 or 70 days of storage.

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 25 mg/ml mixture varied between 23.02 and 26.03 mg/ml [standard deviation (SD) of 1.01], for the 75 mg/ml mixture varied between 71.65 and 82.7 (SD of 2.92), for the 150 mg/ml mixture varied between 143.3 and 163.5 mg/ml (SD of 5.4), and for the 250 mg/ml mixture varied between 248.2 and 282.1 mg/ml (SD of 10.7). The mixtures were considered homogeneous.

The mean concentrations of test material (determined by GC/FID) in the 25, 75, 140 and 250 mg/ml dosing solutions were 99.2, 103, 102 and 106% of target concentrations, respectively. All prepared solutions were analyzed (except for the 150 mg/ml mixture prepared on Day 0 for administration to the male high-dose replacement animals). This mixture was used on Day 0 only.

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), 25, 75 or 250 mg/kg bw/day test material. Due to excessive male mortality, the high dose was reduced on Day 1 to 150 mg/kg bw/day in both males and females. 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 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. Except for holidays, animals were observed for morbidity/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. It 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 -4, 0, 7, and weekly thereafter. Adult females were weighed on Days -4, 0, 7, 14 and 23 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 CO₂ 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 or 25 days after copulation. Females showing no evidence of copulation were euthanized 25 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 2 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 (4 or 5 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 ($p \leq 0.01$), and 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 ($p \leq 0.05$).

Test substance	: The purity of the test material was confirmed by gas chromatography with flame ionization detection (GC/FID) to be 99.3% prior to use and 99.2% at study termination. The structure of the test material was confirmed using mass spectrometry.
Reliability	: (1) valid without restriction OECD Guideline study
Flag 19.04.2004	: Critical study for SIDS endpoint.

(1)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

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
Duration of test	:
Doses	: 25, 75 and 150 mg/kg bw/day
Control group	: yes, concurrent vehicle
NOAEL maternal tox.	: = 150 mg/kg bw
NOAEL teratogen.	: = 150 mg/kg bw
NOAEL Fetotoxicity	: = 150 mg/kg bw
Result	: not developmentally toxic at nonmaternally toxic doses
Method	: other: OECD Guideline 421
Year	: 2004

5. Toxicity

Id 63133-74-4

Date 29.10.2003

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 female rats were treated with 25, 50, 100, 200, 300 or 400 mg/kg bw/day by oral gavage for up to 11 days. One 400 mg/kg bw/day rat was euthanized in extremis after 6 doses. Systemic toxicity was noted at doses \geq 200 mg/kg bw/day.

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 (\pm 2 degrees C) and did not constitute a protocol deviation.

Result : NOEL/LOEL: The NOEL for systemic and developmental toxicity in females was 150 mg/kg bw/day.

Actual dose received: The mean concentrations of test material (determined by GC/FID) in the 25, 75, 150 and 250 mg/ml dosing solutions were 99.2, 103, 102 and 106% of target concentrations, respectively.

Parental data:

Mortality: One control male was found dead on Day 19. One 75 mg/kg bw/day male and one 150 mg/kg bw/day female were euthanized on Days 44 and 20 (respectively) for humane reasons.

Clinical observations:

150 mg/kg bw/day: Reduced activity, vasodilation, rapid respiration, and fur wet with saliva along with transient convulsions (chewing, clonic and/or tonic) and tremors were observed in most males. Most females also showed fur wet with saliva and vasodilation, and a single animal had a single incidence of reduced activity.

75 mg/kg bw/day: Transient vasodilation was observed in all males and one female, which abated the following morning. One male and one female also had a single observation of fur wet with saliva. Reduced activity, unkempt hair coat, urine-stained fur, shallow respiration, decreased fecal volume, porphyrin discharges from the eye and red discoloration of the peri-oral fur were observed in the male that was euthanized.

25 mg/kg bw/day: No clinical observations were noted.

Feed consumption: No differences in feed consumption were noted in treated males. Feed consumption was higher ($p \leq 0.05$) on Day 14 of the pre-mating phase and on Day 7 of gestation in 75 mg/kg bw /day females than controls.

Body weight:

150 mg/kg bw/day: Mean body weights for males were lower ($p \leq 0.05$) than control from Day 7 to study termination. Mean body weight gains were lower ($p < 0.05$) for the males on Days 7 and 25. There was no effect of treatment on body weights or body weight gains in females treated with this or lower doses.

75 mg/kg bw/day: Mean body weights for males were lower ($p < 0.05$)

than control from Day 7 to study termination.). Mean body weight gains were lower ($p < 0.05$) for the males on Days 7.

Organ weights: Mean absolute testes weights were lower ($p < 0.05$) for the 150 mg/kg bw/day group than controls. Relative testes to body weight ratios were similar in treated animals than controls.

Pathology: All of the gross observations were noted in 2 male and 2 female rats. None of them were related to treatment. They were either considered to either represent spontaneous disease processes or gavage error. Two 150 mg/kg bw/day males had minimal seminiferous tubule atrophy (one tubule in each testis), which was within expected background and not considered to be substance-related. All other organs were normal in appearance.

Reproductive and Developmental Data: There was no effect of treatment on any developmental parameter measured (numbers of implants, numbers of corpora lutea, pre and post implantation loss, pup survival, live and dead pups, male and female pups and pup body weight). Clinical observations observed in pups included bruising/hematomas, pups appearing small, pups having little or no milk in stomachs, darker in color, and pale. These observations occurred in similar incidences in treated and control animals. There was no 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 +/- 10.3 g (mean +/- SD) and the females were 67 days of age and weighed 233.6 +/- 9.7 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 36.3 – 54.9%, respectively. Certified feed and water were supplied ad libitum. No contaminants were present in food and water at levels sufficient to affect the outcome. Feeders were changed and sanitized once per week.

Test material: Based on an 11-day probe study in female rats, the doses selected for use in the main study were 25, 75, and 250 mg/kg bw/day. The original high dose of 250 mg/kg bw/day was reduced to 150 mg/kg bw/day in the main study after the first day of dosing because of excessive mortality in male rats (4/12 within 4 hours of dosing). For the low and mid dose groups, the test material mixtures were prepared once based on the stability of the mixture. Changing the dose level for the high dose group to 150 mg/kg bw/day resulted in the mixtures being prepared three times during the study (pre-study, Day 0 and Day 1).

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 25, 75, 150 and 250 mg/ml. Stability of the test material in corn oil was determined by repeated analysis of 25, 75 and 250 mg/ml mixtures of the test substance. Mixtures of 25 mg/ml were analyzed using gas chromatography/flame ionization detection (GC/FID) on 0, 1, 2, 3, 55 and 69 days. Mixtures of 75 and 250 mg/ml were analyzed 0, 1, 2, 3, 4, 7, 14, 28, 51 and 70 days after test mixture preparation. Concentrations of test material were 26.9 +/- 1.53, 76.6 +/-

1.38 and 257 +/- 5.0 mg/ml (mean +/- SD) prior to storage and were 25.8 +/- 0.96, 81.1 +/- 4.02 and 253 +/- 8.5 mg/ml after 69 or 70 days of storage.

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 25 mg/ml mixture varied between 23.02 and 26.03 mg/ml [standard deviation (SD) of 1.01], for the 75 mg/ml mixture varied between 71.65 and 82.7 (SD of 2.92), for the 150 mg/ml mixture varied between 143.3 and 163.5 mg/ml (SD of 5.4), and for the 250 mg/ml mixture varied between 248.2 and 282.1 mg/ml (SD of 10.7). The mixtures were considered homogeneous.

The mean concentrations of test material (determined by GC/FID) in the 25, 75, 140 and 250 mg/ml dosing solutions were 99.2, 103, 102 and 106% of target concentrations, respectively. All prepared solutions were analyzed (except for the 150 mg/ml mixture prepared on Day 0 for administration to the male high-dose replacement animals). This mixture was used on Day 0 only.

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), 25, 75 or 250 mg/kg bw/day test material. Due to excessive male mortality, the high dose was reduced on Day 1 to 150 mg/kg bw/day in both males and females. 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 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. Except for holidays, animals were observed for morbidity/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. It 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 -4, 0, 7, and weekly thereafter. Adult females were weighed on Days -4, 0, 7, 14 and 23 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 CO₂ 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 or 25 days after copulation. Females showing no evidence of copulation were euthanized 25 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 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 (4 or 5 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: 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, 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 ($p \leq 0.01$), and 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 99.3% prior to use and 99.2% at study termination. The structure of the test material was confirmed using mass spectrometry.
 - Reliability** : (1) valid without restriction
OECD Guideline study
- 19.04.2004 (1)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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- (2) Eastman Chemical Company. 2003. Material safety data sheet for N-cyanomethyl-N-ethyl-m-toluidine, dated January 7, 2003.
- (3) Eastman Kodak Company. 1964. Unpublished toxicity report for N-cyanomethyl-N-ethyl-meta-toluidine (Nitrile III), dated May 1, 1964. Notebook number 64, page 89.
- (4) EPIWIN 3.11 Fugacity Level III Model Program.
- (5) EPIWIN AOP Program (v1.91).
- (6) EPIWIN KOWWIN Program (v1.67).
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- (10) Light GC. 2003. N-(cyanomethyl)-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-431-000293-A, dated February 26, 2003.
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